

THE FUNCTIONAL MORPHOLOGY OF THE MOUTHPARTS OF BLACKFLY LARVAE (DIPTERA: SIMULIIDAE)

MARY M. CHANCE

Department of Entomology
University of Alberta
Edmonton, Alberta
Canada

Quaestiones entomologicae
6 : 245-284 1970

Blackfly larvae select their food on the basis of size. Diameters of particles ingested by four filter-feeding species; Cnephia dacotensis Dyar and Shannon, Simulium decorum Walker, Simulium venustum Say, and Simulium vittatum Zetterstedt, range from less than 1 micron to about 350 microns. Most commonly ingested particles ranged from 10-100 microns in diameter. The size distribution of ingested particles varied between species. Larvae of Twinnia biclavata Shewell, a non-filter feeding species, ingests particles of a similar size by grazing. Differences in feeding among filtering species are not attributed to morphological differences. The mouthparts of filtering and grazing species are well adapted for their respective modes of feeding.

Chemical control programs have been aimed at blackfly larvae for over 20 years. Among the most effective formulations is DDT adsorbed on particles which blackfly larvae ingest along with their particulate food (Fredeen, Arnason and Berck, 1953; Fredeen, Arnason, Berck and Rempel, 1953; Noel-Buxton, 1956; Kershaw et al., 1965).

The primary aim of this study was to determine the size of particles which blackfly larvae ingest. This information would be helpful in developing a particulate larvicide which is specific for blackfly larvae and less harmful to other aquatic fauna. The size range of the particles ingested was determined by feeding the larvae variously sized beads. Species variability in feeding was examined in a detailed morphological study of the mouthparts of the species involved.

The most comprehensive morphological studies on head capsules of blackfly larvae are those of Puri (1925), Fortner (1937) and Grenier (1949). More recently Davies (1960), Dumbleton (1962a, b) and Rubtsov (1964) produced detailed works on the larval head capsule and its appendages. Other morphological studies on larval blackflies include those of Debot (1932), Sommerman (1953), and Wood (1963).

Naumann (1924) and Fortner (1937) also studied filter-feeding by blackfly larvae. The most recent review of filter-feeding is that of Jørgensen (1966), who was concerned mainly with marine invertebrates but also included most of the work done on insects. Pucat (1965) studied filtering by mosquito larvae. Burt (1940) and Walshe (1947, 1951) studied filtering by midge larvae.

Among the most important studies on the biology of larval blackflies are those of Wu (1931), Grenier (1949), Peterson (1956), Zahar (1957), Davies and Syme (1958), Anderson and Dicke (1960), Carlsson (1962, 1967), Phelps and DeFoliart (1964), Rubtsov (1964) and Maitland and Penny (1967). Feeding habits are also discussed by Smart (1944), Badcock (1949), Jones (1949a, b, 1950, 1951, 1958), Fredeen (1960, 1964), Williams et al. (1961) and Abdelnur (1968). I have reviewed (*Quaest. ent.*, in press) control programs aimed at blackfly larvae.

MATERIALS AND METHODS

Larvae of *Cnephia dacotensis* Dyar and Shannon, *Simulium decorum* Walker, *Simulium venustum* Say or *Simulium verecundum* Stone and Jamnback, and *Simulium vittatum* Zett., were reared in the laboratory. It was not practical to separate larvae of *S. venustum* and *S. verecundum* and these are treated here as *S. venustum*. Larvae of *Prosimulium* species, mainly *P. travisi* Stone, and larvae of *Twinnia biclavata* Shewell were also collected.

Three methods were used to rear larvae in the laboratory; all of them were closed systems. Two were stream methods and were not as successful as the third, a jar method similar to that used by Puri (1925) and Davies and Smith (1958). Several battery jars were filled with 4 to 8 litres of a mixture of tapwater and deionized water. Air breaker stones in each jar produced the necessary water movement and aeration. The air was taken from a laboratory air supply of gauge pressure of 1.7 to 2.0 atm. and passed through an oil trap and charcoal filter. At the bottom of each jar there was a layer of charcoal 4 to 6 cm deep. The temperature of the water varied with the room temperature from 22 to 27 C. Larvae were fed on bakers' yeast. Organic material which was gathered during collections and which accumulated in the jars was also available to the larvae. Blackflies were reared from egg to adult by this method.

Live larvae were observed through a stereomicroscope through the wall of a containing vessel. Larvae were also observed while attached to a thin plastic or glass plate inverted over a container of water.

Specimens were preserved in 90% ethanol or 1:3 glacial acetic acid and 90% ethanol. Whole mounts of head capsules in Canada Balsam were also examined. Most of the head structures could be studied without any special preparation, especially in recently moulted specimens. For detailed study, specimens were treated with a solution of 4% potassium hydroxide at room temperature for 24 hours. Borax carmine was used to stain muscles. Mallory's triple stain (Pantin, 1960; modification by Sharplin, *personal communication*) was used for studying cuticular structures. Serial sections of heads stained in Ehrlich's haematoxylin and eosin were also studied.

To determine the size range of particles ingested, blackfly larvae were exposed to four types of 'Sephadex' beads (Pharmacia Fine Chemicals Inc.): 'G-25 superfine', 'G-25 fine', 'G-100' and 'G-200'. Diameters of beads after swelling ranged from 10 to 445 microns. The beads were swollen according to their individual requirements ('Sephadex' booklet No. 2, Theory and Experimental Technique).

The samples of 'Sephadex' to which the larvae were exposed were made up of equal volumes (17.5 ml) of the four types of 'Sephadex'. After varying lengths of time (10 min to 2 hr), larvae were removed from the jars and their guts examined. To determine the frequency distribution of sizes of beads available to the larvae, 500 randomly selected beads from each of five exposure samples (also made up of equal parts of the four 'Sephadex' types) were measured.

Larvae were classified into three age groups: small, medium and large, using a technique similar to that of Phelps and DeFoliart (1964). The categories are defined on the basis of the length of the cephalic apotome, the width of the head capsule at its widest part, and the degree of development of the pupal histoblast. Consideration of the histoblast was subjective. Small larvae have no readily visible histoblasts. Large larvae have coloured or large white histoblasts in which the respiratory filaments are well differentiated. Medium larvae have histoblasts of intermediate development. The lengths and widths of the head capsules of the four filtering species of blackfly larvae are tabulated below (Table 1).

Table 1. Size in microns of head capsules of small, medium and large larvae of four species of blackflies.

No small larvae of *S. decorum* or *S. venustum* were available for measurement.

Species	Small		Medium		Large	
	length	width	length	width	length	width
<i>C. dacotensis</i>	140-519	120-319	520-759	320-539	760-1159	540-779
<i>S. decorum</i>	-599	-399	600-719	400-519	720- 919	480-559
<i>S. venustum</i>	-699	-519	700-799	520-599	800- 899	640-719
<i>S. vittatum</i>	140-499	80-379	500-819	380-659	820-1039	660-799

MORPHOLOGY OF THE MOUTHPARTS AND RELATED STRUCTURES

Filter-feeding species

The head capsule of blackfly larvae is subcylindrical, and tapers towards the cervical region (Fig. 1). It is prognathous. The cephalic cleavage lines (c. c. l.) are roughly parallel in well-developed larvae but converge at the midline of the posterodorsal margin of the head in first instars.

The ventral wall of the head capsule has recently been considered part of the head capsule and is referred to as the hypostomium (hypo., Fig. 2, 3) (Grenier, 1949; Crosskey, 1960; Wood, 1963; Dumbleton, 1964; Davies, 1965). Since the terminology used in this study follows closely that of Crosskey (1960), this term is adopted here. However, Matsuda (1965) describes the tendency for the submentum of insects to become sclerotized and to fuse with the gular region of the prognathous head. The posterior margin of the submentum is then recognized by the origins of the submento-mental muscles which lie on the line connecting the posterior tentorial pits. This being so, the ventral wall of the blackfly larval head is submental. Craig's (1969) embryological study supports this relationship.

The head spots (hd. spot, Fig. 1) mark the origins of the cephalic muscles. The antennae (ant., Fig. 1, 3) are at the anterolateral corners of the cephalic apotome. In late instar larvae they have four articles and bear two sensory papillae on the apex of the second article. In early instar larvae the antennae have two or three articles.

The origin of the pair of food collecting organs, the cephalic fans (c. fan, Fig. 1 to 3), is still controversial. On the basis of conflicting theories they have been considered messorial (Cook, 1949), from the Latin term *messor*, meaning reaper, first applied to structures of the head capsule of mosquito larvae (Cook, 1944). This concept has been rejected by most as being erroneous (Snodgrass, 1959; Chaudonneret, 1962, 1963). Puri (1925) and others (Fortner, 1937; Grenier, 1949; Craig, 1969) considered the cephalic fans premandibular, and Crosskey (1960) and others (Wood, 1963; Davies, 1965) considered them labral. They have been termed mouth-brushes (Smart, 1944; Dumbleton, 1962b) and cephalic fans (Puri, 1925; Crosskey, 1960; Dumbleton, 1962a). Since 'fan' is a more accurate description and since they are not members of the typical insect mouthparts, I prefer to call them cephalic fans.

Cephalic fans. — The cephalic fans (Fig. 1 to 3) are paired structures arising from the anterolateral corners of the cephalic apotome adjacent to a pair of unpigmented knobs (Fig. 1, 3). This is the first time these knobs have been reported. The dorsal surface of the cephalic fan stem is sclerotized and consists of two large sclerites which Puri (1925) considered segments. The larger sclerite, called P1 (= *large plaque*, after Grenier 1949, Fig. 4, 54) forms most of the dorsal surface and articulates with the head capsule. The upper third

of the sclerite bears three to six sensory hairs; one large hair is present close to the tip of the stem. The second sclerite, Pb (= *plaque basal*, after Grenier 1949) is spindle-shaped and horizontally arranged. It lies basal to Pl, lateral of the stem.

The ventral wall of the stem is concave and membranous. It supports three well-developed fans (Fig. 4). The primary fan (p. f.) arises from the apex of the stem; the secondary fan (s. f.), elsewhere called the accessory fan (Grenier, 1949), and the basal fan (Rubtsov, 1964), lies laterobasal to the primary fan; the medial fan (m. f.), elsewhere called the marginal fan (Fortner, 1937; Grenier, 1949) and the small fan (Rubtsov, 1964), lies on the medial side of the stem.

The distal half of the ventral wall is reinforced by a strongly sclerotized rod, Sc₁ (after Grenier, 1949) (Fig. 5). Some workers consider this rod to represent part of the torma (Wood, 1963; Wood et al., 1963). It consists of two parts: a ventral, rectangular piece and a dorsal bar. When viewed from the side (Fig. 54) it resembles a 'T', the medial, rectangular part forming the stem. When viewed from the ventral surface, it appears to be highly ridged as described by Grenier. The dorsal bar lies inside the stem and articulates basally with another rod, Scb (after Grenier, 1949). The apex of Sc₁ extends beyond the base of the secondary fan and spreads out laterally to form a fulcrum (ext. Sc₁, Fig. 5) for the rotation of the primary fan rays. Wood (1963) called this extension the connective sclerite in larvae of *Cnephia strenua* MacKerras and MacKerras, and *Simulium pictipes* Hagen. In the species studied here it neither connects directly with the rays and has neither a well-defined border nor division from Sc₁. I consider it an extension of Sc₁. The second rod Scb (Fig. 5) is at right angles to Sc₁, passing from articulation with Sc₁ to the ventrolateral wall between the two dorsal sclerites. It forms the ventrobasal wall of the stem. A third, thinner rod Scm (Fig. 5) (after Grenier, 1949) supports the medial lobe.

The retractor muscle of the cephalic fan inserts on the base of Sc₁ and is composed of three bundles. Two originate on the posterior region of the cephalic apotome, the precise spot being marked externally by the posterolateral head spots. These two bundles interdigitate with those from the other side before passing anteriorly. The third and smallest bundle originates on the midline of the cephalic apotome at the posteriomedial head spot, close to the posterior margin of the head capsule.

The rays of the primary fan are arranged in semicircle around the apex of the stem. When fully expanded, the rays cover an angle of between 200 and 250 degrees. The individual rays are sickle-shaped and hollow. Their bases are expanded into vanes of flexible, membranous cuticle. The shape of the basal expansion varies with the position of the ray in the fan (Fig. 8 to 10). The rays of the lateral side of the fan have slender, narrow expansions; the medial rays have acute, wide, basal expansions.

The numbers of rays of each fan vary with instar and with species. The numbers of rays in each of the three fans of the four filtering species studied are listed in Table 2.

The primary rays have microtrichia on their inner curved surface. The pattern of trichiation varies within the fan and with species. The more lateral rays have fewer microtrichia than the medial ones and the microtrichia are more numerous midway along rays. The larvae of both *C. dacotensis* and *S. vittatum* have no pattern of trichiation (Fig. 11, 15); however, among *C. dacotensis* larvae the microtrichia occur irregularly in two rows along the concave surface of the ray. The primary rays of *S. decorum* larvae have no pattern of trichiation but the arrangement of one long, two short, one long is sporadically repeated (Fig. 16). The trichiation of *S. venustum* larvae is arranged in a pattern similar to that of *Prosimulium* species studied (Fig. 17).

The bases of the secondary fan rays lie in a curved line (Fig. 7). This base line connects with that of the primary rays along a row of approximately 10 blades (Fig. 4, 5, not all

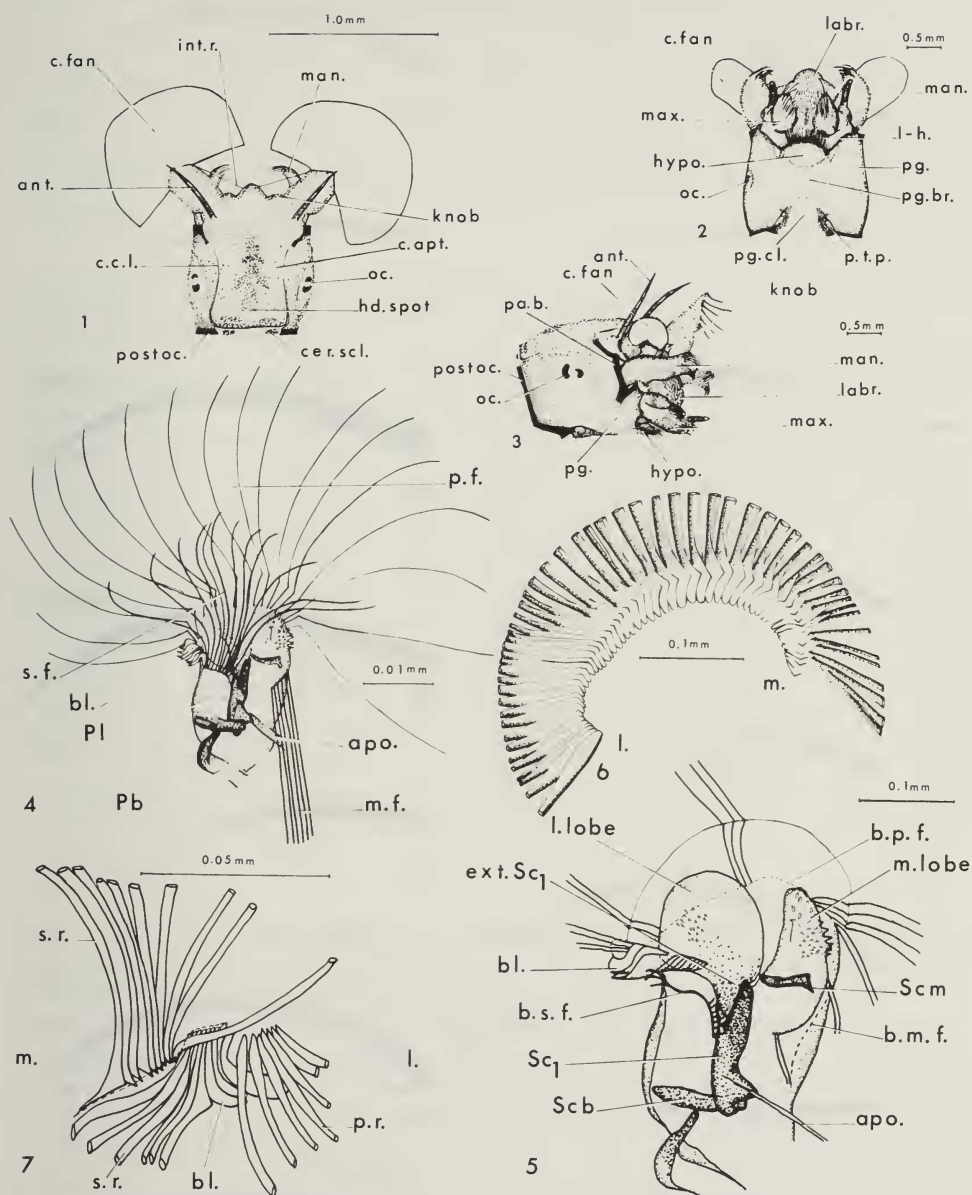


Fig. 1-7. Fig. 1-3: Head capsule of *S. vittatum* larva. 1, dorsal view; 2, ventral view; 3, lateral view. Fig. 4-7: Cephalic fan of *S. vittatum* larva. 4, ventral view; 5, ventral view of stem; 6, primary fan base; 7, secondary fan base. ant. = antenna, apo. = apodeme, bl. = blades, b. m. f. = base of medial fan, b. p. f. = base of primary fan, b. s. f. = base of secondary fan, c. apt. = cephalic apotome, c. c. l. = cephalic cleavage lines, cer. scl. = cervical sclerite, c. fan = cephalic fan, ext. Sc₁ = extension of Sc₁, hd. spot = head spot, hypo. = hypostomium, int. r. = intermediate ray, l. = lateral, labr. = labrum, l-h. = labio-hypopharyngeal complex, l. lobe = lateral lobe, m. = medial, man. = mandible, max. = maxilla, m. f. = medial fan, m. lobe = medial lobe, oc. = ocelli, pa. b. = postantennal buttress, Pb = dorsal sclerite of fan stem, p. f. = primary fan, pg. = postgena, pg. br. = postgenal bridge, pg. cl. = postgenal cleft, Pl = dorsal sclerite of cephalic fan stem, postoc. = postocciput, p. r. = primary ray, p. t. p. = posterior tentorial pits, Sc₁ = sclerite of fan stem, s. f. = secondary fan, s. r. = secondary ray.

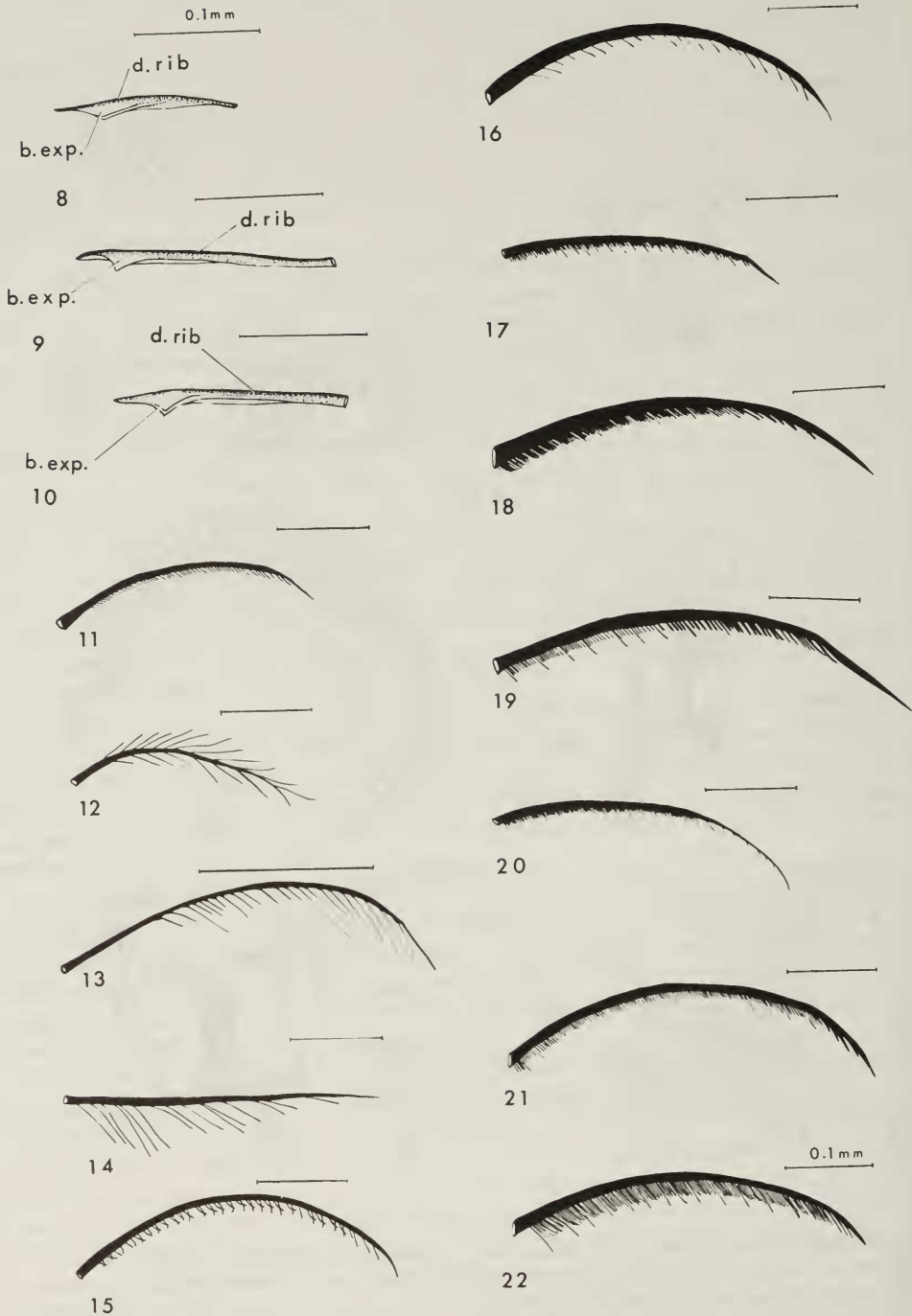


Fig. 8-22. Fig. 8-10: Bases of primary rays of *S. vittatum* larva. 8, lateral ray; 9, mid-fan ray; 10, medial ray. 11, primary ray of *S. vittatum*; 12, secondary ray of *S. vittatum*; 13, intermediate ray of *S. vittatum*. 14, medial ray of *C. dacotensis*. Fig. 15-22: primary rays. 15, *C. dacotensis*; 16, *S. decorum*; 17, *S. venustum*; 18, *P. fontanantum*; 19, *P. frohnei*; 20, *P. fuscum*; 21, *P. multidentatum*; 22, *P. travisi*. b. exp. = basal expansion, d. rib = dorsal rib.

blades are represented in Fig. 7). These decrease in size towards the secondary fan. These blades probably represent degenerate rays. The curve of the base line of the secondary rays contributes to the whirling of the rays during opening and closing of the secondary fan. When the fan is fully expanded, the rays cover an angle of about 270 degrees. They overlie the basal quarter of the medial primary rays. Lacking a dorsal rib, the secondary rays are weaker than the primary rays. The bases of the individual rays are triangular, similar to those of the primary rays, but there is less variation in the basal expansions. Unlike the bases of the primary rays, the bases of the secondary rays are flexible. The secondary ray itself is rigid.

Table 2. Numbers of rays in the three fans of larvae of four species of blackflies at three stages of development.

Size of larvae	Species	Primary	Secondary	Medial
Large	<i>C. dacotensis</i>	47-56	23-35	10-15
	<i>S. decorum</i>	54-64	29-39	9-15
	<i>S. venustum</i>	34-45	12-28	8-12
	<i>S. vittatum</i>	42-54	20-30	9-14
Medium	<i>C. dacotensis</i>	39-50	22-32	7-12
	<i>S. decorum</i>	44-54	16-35	6-12
	<i>S. venustum</i>	41-54	20-29	9-13
	<i>S. vittatum</i>	42-54	11-29	6-11
Small	<i>C. dacotensis</i>	19-32	6-14	2- 8
	<i>S. decorum</i>	44-46	16-22	6-10
	<i>S. venustum</i>	18-36	10-25	5- 9
	<i>S. vittatum</i>	27-46	9-16	4- 7

The trichiation of the secondary fan is similar in all species, however, the secondary rays of *S. decorum* larvae are crescent-shaped. Secondary rays bear microtrichia on their ventral and lateral surfaces (Fig. 12). The microtrichia are longer and denser than those of the primary rays and they form an acute angle with the ray, giving it a plumose appearance.

The medial rays differ from the rays of the other fans in that they lie in a straight line, are not curved, and have bulbous bases. The rays lie parallel to each other and do not spread out when the fan is opened. The rays are flexible and the bases are membranous.

The medial rays of *C. dacotensis* (Fig. 14) and *S. vittatum* bear microtrichia. These are sparse and arise from small notches on one side of the ray. Larvae of *S. decorum* and *S. venustum* do not have microtrichia on the medial rays. This situation is found among other species (Fortner, 1937; Rubtsov, 1964).

The single intermediate ray is present in all age groups of larvae of all species studied. It is located between the primary and medial fans, adjacent to the most lateral primary ray. It resembles the medial rays of *C. dacotensis* and *S. vittatum* in trichiation and is straight and colourless (Fig. 13).

In the first instars of *C. dacotensis* and *S. vittatum* the cephalic fan is functional but only the primary fan is present. The primary rays have the same basal expansion found in later instars but have no microtrichia. Neither the medial nor the lateral lobe is present. The

rod Sc_1 is present and has the expanded distal tip. It bears the apodeme of the cephalic fan muscle. The structure of the cephalic fan of *S. pictipes* first instar, described by Wood (1963), is very similar to that of these two species.

The cephalic fan of the second instar of both *C. dacotensis* and *S. vittatum* is fully formed.

The dimensions of the primary fans vary between species and between larvae of different ages. The midfan widths of the open fan are tabulated below for the four species studied (Table 3). The frontal area (F. A.) of the fan was calculated from the modified equation for the area of an ellipse: $F. A. = \frac{ab\pi}{4}$ where, a = width of the fan, b = depth of the fan.

Table 3. Dimension in microns of the expanded primary fan of four species of blackfly larvae at three stages of development.

Size of larvae	Species	Width	Depth	Frontal Area (mm ²)
Large	<i>C. dacotensis</i>	1020-1200	420-540	0.34-0.51
	<i>S. decorum</i>	800-1060	240-340	0.15-0.26
	<i>S. venustum</i>	800- 880	260-420	0.16-0.29
	<i>S. vittatum</i>	890-1120	380-540	0.17-0.48
Medium	<i>C. dacotensis</i>	380- 700	140-380	0.04-0.21
	<i>S. decorum</i>	-	-	-
	<i>S. venustum</i>	460- 720	200-320	0.07-0.18
	<i>S. vittatum</i>	780- 850	360-460	0.22-0.31
Small	<i>C. dacotensis</i>	260- 340	80-160	0.02-0.04
	<i>S. decorum</i>	-	-	-
	<i>S. venustum</i>	200- 400	80-200	0.01-0.06
	<i>S. vittatum</i>	460- 560	200-260	0.08-0.12

The cephalic fans of the *Prosimulium* species examined differ from those already described in several probably functionally insignificant ways. Because of its diagnostic value, the arrangement of the rays of the secondary fan is important. When the fan expands, the apices of the ray lie in a straight line. This feature differentiates *Prosimulium* species, as well as some *Gigantodax* and *Cnephia* species (Wood, 1963), from other genera of blackflies (Sommermann, 1953). The arrangement of the bases of the secondary rays, in addition to the length of the rays, contributes to this distinction. Wood (1963) stated that the number of rays and the length of the base line of the secondary fan is a fundamental difference between the secondary fan of *Prosimulium* species and that of other species of blackflies.

The secondary fan of *Prosimulium* larvae is separated from the primary one by about six blades. These are of equal size. The separation between medial and primary fans is obscured by four to six large rays all of which resemble the intermediate ray. Wood (1963) represented only one such ray for *Prosimulium fontanatum* Syme and Davies larvae. The medial rays lack microtrichia.

In general, the trichiation of *P. fontanatum*, *Prosimulium frohnei* Sommermann, *Prosimulium fuscum* Syme and Davies, *Prosimulium multidentatum* Twinn and *Prosimulium travisi* Stone resembles that of *S. venustum*, but the pattern of trichiation of the primary rays is more pronounced (Fig. 18 to 22). Differences of pattern of trichiation are specific.

Labrum. — The labrum in all species studied was very similar. It is a beak-shaped structure overhanging the cibarium (cib., Fig. 56), and is joined to the cephalic apotome by a membranous area. This area (mem. ar., Fig. 23, 24) lacks bristles but is provided with numerous sensory hairs. In *S. vittatum* larvae the margin of the cephalic apotome immediately dorsal to the membrane has three patterns of pigmentation (Fig. 25): a straight border, a border with a small indentation in the midline, or a border with a protruding central lobe.

The posterior margin of the labrum is marked by a single line of well-developed simple bristles with bulbous bases (Fig. 23). Behind this line there is a medial pair of sensory hairs. The posterior margin of the main bristled area of the labrum is marked by another straight line of bristles; these have triangular bases. The main bristled area of the labrum is covered by shorter bristles which occur in groups of two to four. In the midline towards the apex of the labrum there is a spindle-shaped patch of stout, blunt, conical spines (c. sp. br., Fig. 23) which are located on an elevated base. In *P. fontanatum* and *P. multidentatum*, they are poorly developed; in *P. frohnei*, they are dorsally located. In other simuliids these spines have been described as labral hooks similar to those of the thoracic proleg and the posterior disc (Hora, 1930), and as pectinate hairs (Grenier, 1949).

The labrum is strengthened by a spade-shaped sclerite (labr. scl., Fig. 24, 26). Some authors have considered this sclerite to consist of three sclerites (Puri, 1925; Wood, 1963; Rubtsov, 1964 and others). Rubtsov stated that each sclerite bears a brush; this was not found among the species studied. Other workers consider the sclerite as a unit (Davies, 1964). The latter interpretation is accepted here as no sutures are evident. However, in the following description the sclerite is considered in three sections; the apex (ax.), the connecting rod (conn. r.) and the basal piece (b. p., Fig. 26). The basal piece is at right angles to the connecting rod on the same plane. The apex of the sclerite forms the tip of the ventral wall. The anterior margin of the apex is dentate (Fig. 26). The teeth are peg-shaped, usually of equal length and of varying widths. However, the lateral teeth of the labral sclerite of *P. travisi* larvae are curved and longer than their fellows. In the larva of an unidentified species of blackfly, four of these teeth, two medial and two lateral, have neural connections (D. A. Craig, *personal communication*), showing that the teeth are sensory. The lateral borders of the sclerite are composed of three to five sclerotized blades (l. bl.) each of which bears several bristles arranged in a row (Fig. 26). The basal piece of the sclerite passes inwards and supports the epipharynx (epi., Fig. 56). Ventrally the connecting rod supports a lobe (v. lobe) bearing a dense brush of long, thick, compound bristles (Fig. 24). Grenier (1949) considered this lobe to be the epipharynx.

The cuticle of the labrum is flexible. The bristles are stiff although the shorter, central ones of the main bristled area are more flexible than the others. The apex of the labral sclerite is of more rigid cuticle.

The labrum has only one pair of muscles, the labral retractors (labr. r., Fig. 24). All but one pair of labral retractors have been lost in other nematoceros larvae as well (Hinton, 1958a; Chaudonneret, 1963). In simuliids the retractors insert on the ventral surface of the labral sclerite where the apex joins the connecting rod. They originate at the anteromedian head spot in the midline of the cephalic apotome. Contraction of the labral retractors moves the labrum ventrally and orally. Chaudonneret (1963) stated that the elasticity of the cuticle and the pressure of the internal environment play an antagonistic role to that of the labral muscles. The cavity of the labrum is filled by a pair of dorsal glands (d. gl., Fig. 56) which open directly into the cibarium.

Midway between the labrum and the stem of the cephalic fan there is a patch of compound bristles (Fig. 24). The number, from 15 to 50, and colour vary with species.

Mandibles. — The mandibles are broadly rectangular and flattened laterally (Fig. 27, 28). They are curved medially and bear brushes on their concave surface. The mandibular articulations have changed position from those of the primitive insect, a trend found among other

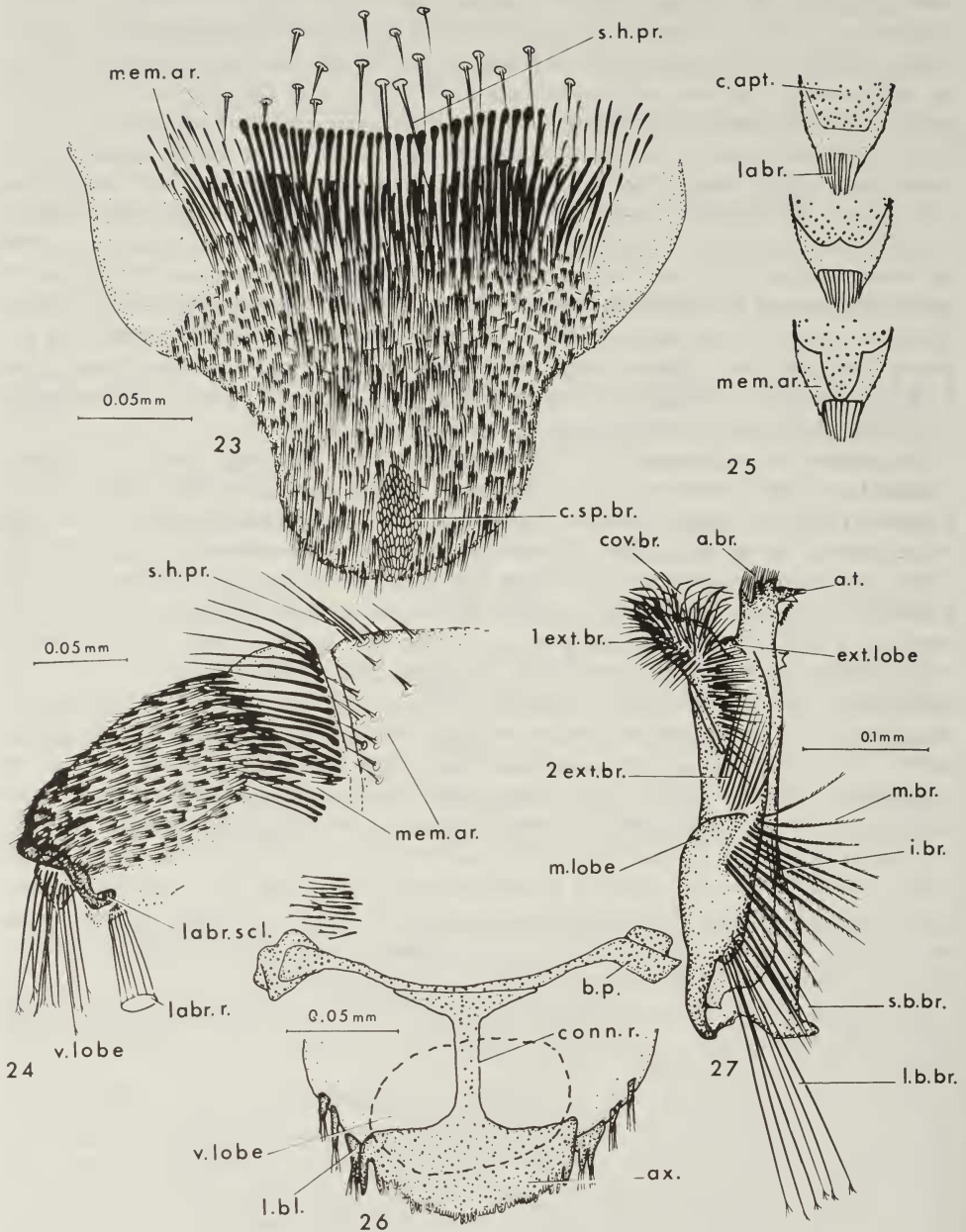


Fig. 23-27. Fig. 23-26, Labrum of *S. vittatum*. 23, dorsal view; 24, lateral view; 25, pigmentation of the anterior of cephalic apotome, not to scale; 26, labral sclerite. 27, mandible of *S. vittatum*, inner view. a. br. = apical brush, a. t. = apical teeth, ax. = apex of labral sclerite, b. p. = basal piece, c. apt. = cephalic apotome, conn. r. = connecting rod, cov. br. = covering brush, c. sp. br. = conical spine-like bristles, 1 ext. br. = first external brush, 2 ext. br. = second external brush, ext. lobe = external lobe, i. br. = inner brush, labr. = labrum, labr. r. = labral retractor muscle, labr. scl. = labral sclerite, l. b. br. = large basal brush, l. bl. = lateral blade, m. br. = middle brush, mem. ar. = membranous area, m. lobe = middle lobe, s. b. br. = small basal brush, s. h. pr. = pair of sensory hairs, v. lobe = ventral lobe.

Nematocera (Cook, 1949). The mandibles of blackfly larvae articulate in sockets formed by strong, heavily sclerotized X-shaped structures, the postantennal buttresses (pa. b., Fig. 3). Two ventrally-directed arms of each buttress provide a pivot for the base of the lateral sides of the mandible. The anterior dorsal arm of the buttress supports the base of the wall of the cephalic fan; the fourth arm passes ventral to the antenna. With its points of articulation midway along the ventral arm of the buttress, the mandible moves in a plane forming an angle of 30 to 40 degrees with the vertical plane through the longitudinal axis of the body. This angle is subsequently called the angle of articulation.

The base of the mandible is strengthened by a thick, strongly sclerotized ridge which follows a longitudinal cleft in the medioventral surface of the mandible. The apodeme of the retractor muscle inserts at the apex of this cleft. The extensor muscle inserts directly opposite at the base of the adoral surface. Both muscles originate at the posterior margin of the postgenae. The extensor consists of five bundles; the retractor consists of three bundles.

The mandible bears three sets of teeth (Fig. 29, 30). There are usually four large, black, heavily sclerotized apical teeth (a. t.), of which three are orientated in different directions with the fourth immediately above these three. *P. frohnei* and *P. travisi* larvae have a fifth tooth which lies adjacent to the fourth. The small, pale inner teeth (i. t.) vary in number between instar and species (Fig. 29, 30). The number and orientation of the marginal teeth (m. t.) vary with species. In *S. vittatum* larvae there are two apically directed teeth; the basal one is the smaller one. *S. venustum* larvae have two to three marginal teeth at right angles to the edge of the mandible. The two marginal teeth of *C. dacotensis* larvae may point either apically or basally. *Prosimulium* larvae have 8 to 14 marginal teeth (Fig. 30). Some of the marginal teeth of *P. frohnei* and *P. travisi* have compound apices.

The mandible bears eight brushes (Fig. 27, 28). The apical brush (a. br.) is made up of rows of short, fine bristles of equal length. They are arranged on small lobes and curve towards the apical teeth. The first external brush (1 ext. br.) stretches from the apex of the mandible to the middle lobe (m. lobe), midway along the concave surface. It consists of numerous, fine bristles with relatively rigid bases. The second external brush (2 ext. br.) arises from the base of the middle lobe. Its few long bristles are directed apically. The middle brush (m. br.) is fan-shaped and its bristles bear microtrichia. Basal to the middle brush there is a small basal brush (s. b. br.) consisting of fine bristles. The inner brush (i. br.), consisting of three to five thick bristles, arises from the apex of the mandibular cleft and is apically directed.

The covering brush (cov. br.) arises distal and medial to the external lobe (ext. lobe) (Fig. 27) located at the apex of the mandible (Fig. 29). The bristles are compound and arise from individual lobes. In contrast to other compound bristles, these branch close to their bases. Both covering brush and the first external brush deflect away from the mandible and so describe a 'V' with the apical teeth (Fig. 27, 52). This deflection and the curve of the bristles permit the two apical brushes to curve over the retracted primary cephalic fan.

The large basal brush (l. b. br.) is the second brush composed of compound bristles. Rubtsov (1964) divided this brush into two parts: large basal bristles and small basal bristles. However, here the 10 to 15 bristles are all long and straight.

The mandible bears small, sensory hairs scattered over both oral and adoral surfaces. There is a pair of large sensory hairs on the apical surface immediately behind the first external brush (Fig. 28). Small spines are present on the dorsobasal corner and on the apical lobe. The basal spines of *S. decorum* larvae are arranged in rows. A large apical spine is present on the ventral surface just behind the inner teeth. In *C. dacotensis* and *S. venustum* larvae this spine is oddly twisted. *Prosimulium* species lack the spine.

To compare the size of the mandible of the three age groups of the four species, three

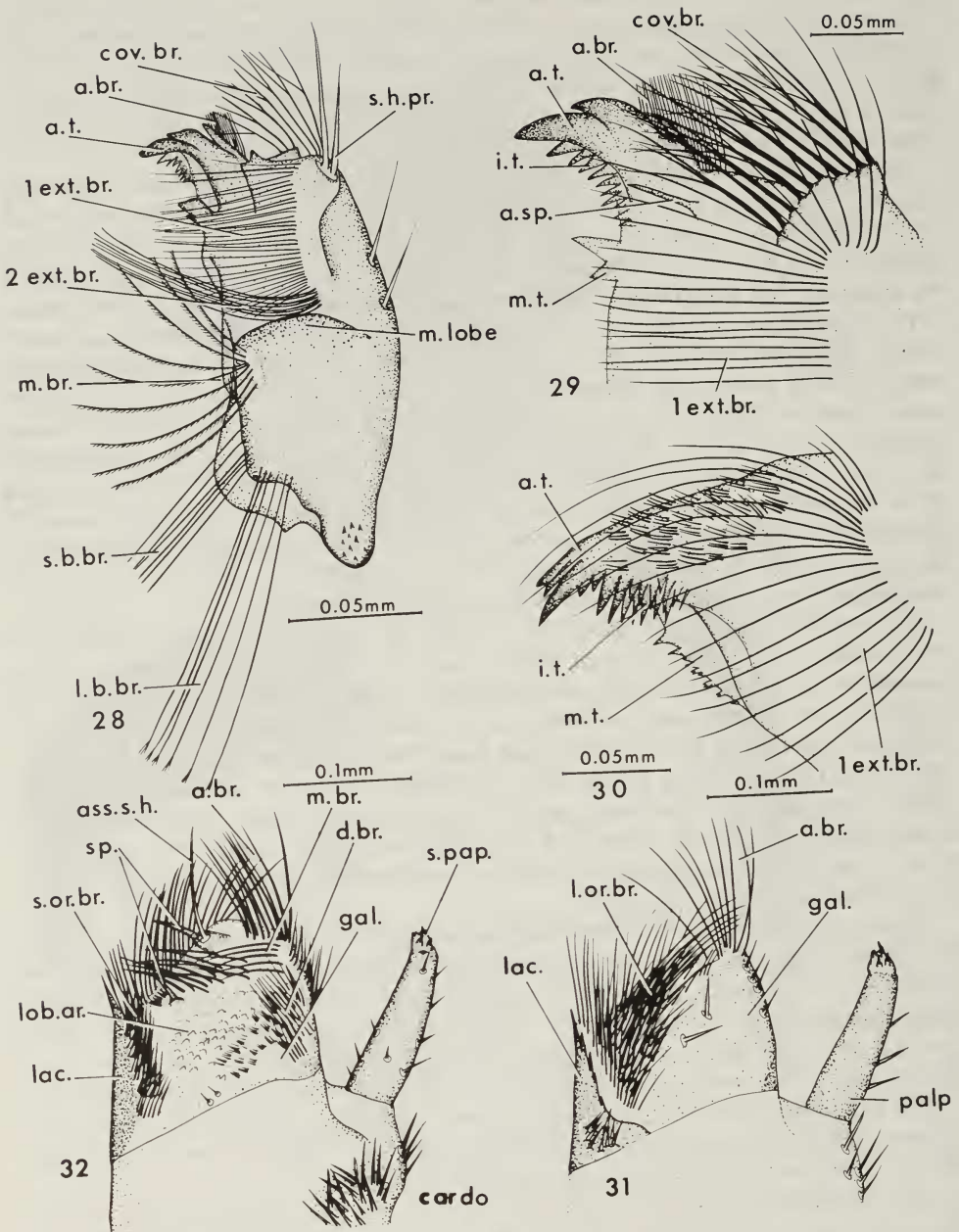


Fig. 28-32. 28, Mandible of *S. vittatum*, lateral view; 29, apex of mandible of *S. vittatum*; 30, apex of mandible of *P. travi* larva; 31, right maxilla of *S. vittatum*, dorsal view; 32, left maxilla of *S. vittatum*, ventral view. a. br. = apical brush, a. sp. = apical spine, ass. s. h. = associated sensory hair, a. t. = apical teeth, cov. br. = covering brush, d. br. = diffuse brush, 1 ext. br. = first external brush, 2 ext. br. = second external brush, m. br. = middle brush, m. lobe = middle lobe, m. t. = marginal teeth, s. b. br. = small basal brush, s. h. pr. = pair of sensory hairs, s. or. br. = small oral brush, sp. = spine, s. pap. = sensory papillae.

parameters were measured: (1) the distance between the apex of the covering brush and the base of the second external brush, (2) the distance between the apex of the covering brush and the base of the middle brush, (3) the distance between the apex of the covering brush and the base of the large basal brush (Table 4). Measurements were made on five larvae in each category of each species.

Table 4. Distance in microns between the covering brush 'c', and the base of the second external brush 'e', the middle brush 'm', and the large basal brush 'lb' of the mandibles of four species of blackfly larvae at three stages of development.

Size of larvae	Species	c-e	c-m	c-lb
Large	<i>C. dacotensis</i>	140-180	180-200	220-310
	<i>S. decorum</i>	150-180	160-190	220-280
	<i>S. venustum</i>	130-150	130-180	220-280
	<i>S. vittatum</i>	140-150	160-180	140-290
Medium	<i>C. dacotensis</i>	50-100	50-120	80-180
	<i>S. decorum</i>	-	-	-
	<i>S. venustum</i>	70-120	90-140	140-220
	<i>S. vittatum</i>	100-120	120-140	180-220
Small	<i>C. dacotensis</i>	40- 50	50- 60	80-110
	<i>S. decorum</i>	-	-	-
	<i>S. venustum</i>	40- 60	50- 80	90-130
	<i>S. vittatum</i>	70- 80	80-100	120-150

The mandibles of the first instar of *S. vittatum* have an almost complete set of teeth and brushes. However, there are no marginal teeth and the large basal brush is represented by only one bristle. The only other anatomical difference is the position of the pair of sensory hairs on the apex. In first instars it arises in front of the first external brush rather than behind as in later instars. The mandible of the second instar has a similar smaller number of component parts but the pair of sensory hairs is behind the first external brush.

On some *S. vittatum* larvae the bristles of the large basal brush have a globular structure which has not been described elsewhere (Fig. 55). These bulbs are always found on the bristles at the region where they branch. Usually two or three bristles in one brush each have a bulb. On later instars the bulbs are darkly coloured; on younger larvae they are pale. The bulbs turn pink and subsequently become colourless when treated with a 4% solution of potassium hydroxide but they do not dissolve. The colour returns when the larvae are returned to alcohol. I have observed the bulbs preserved in 90% ethanol, 70% ethanol after Bouin's fixative, and in a mixture of glacial acetic acid and 70% ethanol. I have also observed them on specimens mounted in Canada balsam after exposure to alcohols and xylene. Larvae bearing these bulbs have been found in one population from Ontario (railway yards, Belleville) and two populations in Alberta (Johnson Lake inlet, Banff National Park; Whitemud Creek, Edmonton). The proportion of larvae of the Ontario population is tabulated below (Table 5). A few larvae, about 5%, of the Johnson Lake collections had bulbs. One specimen of *P. travisi* collected in Alberta had a bulbous structure resembling those on the mandible of *S. vittatum* larvae. It was on a large basal bristle. Two specimens of *S. vittatum* had similar bulbs on simple labral bristles.

Table 5. Proportion of *S. vittatum* larvae from an Ontario population bearing bulbs on large basal bristles of the mandibles.

Size of larvae	On both mandibles		On one mandible		No bulbs		Total	
	no.	%	no.	%	no.	%	no.	%
Large	16	45.7	4	11.4	15	42.9	35	100.0
Medium	10	28.6	4	11.4	21	60.0	35	100.0
Small	3	8.6	2	5.7	25	71.4	30	100.0
No. exam.	29	29.0	10	10.0	61	61.0	100	100.0

These bulbs are not a fungus (H. T. Brodie, Department of Botany, University of Alberta, *personal communication*) neither are they a particle impaled by the bristles. They may be a swelling of the apices of the bristles or some type of sensory organ.

Maxillae. — The maxilla of blackfly larvae is mitten-shaped, the maxillary palp representing the thumb (Fig. 31, 32). The structure of the maxillae is consistent among all the species studied. The maxillae lie ventral to the mandibles and dorsolateral to the labiohypopharyngeal complex. The palp is aboral.

The maxilla is sclerotized in three areas which Rubtsov (1964) considered as the cardo, lacinia and galea. According to Cook (1949), the maxilla is reduced and consists of the stipes which is partly sclerotized and partly membranous. The sclerotized areas are of relatively inflexible cuticle; the other areas, except the pigmented area of the palp, are of flexible cuticle.

The maxillary lobe bears five brushes. The ventral adoral surface bears a diffuse brush (d. br.) composed of fine, randomly-arranged bristles. Medial to this brush, on the apical half of the lobe, the middle brush (m. br.) is similarly composed of fine, simple bristles. A large oral brush (l. or. br.) lies adjacent to the middle brush. This brush is composed of 10 to 15 rows each containing 12 to 15 long, thick and darkly-pigmented bristles. Laterobasal to the large oral brush lies a small oral brush (s. or. br.) similarly composed of bristles arranged in rows. The apical brush (a. br.) consists of six to ten simple bristles which are long and thick and have expanded bases.

On its distal border, the lacinia bears a row of teeth which increase in size towards the apex of the lacinia. The most distal tooth is spine-like, long and curved at its apex. The lacinia has one small patch of bristles on the corner adjacent to the small oral brush. The galea bears a large, central sensory hair and five to eight small apical hairs.

Basal to the middle brush there is a bare area with a lobulate surface (lob. ar.). These lobes are present on all specimens and have been described elsewhere (Rubtsov, 1964).

The maxillary lobe bears two large sensory spines (sp.) (D. A. Craig, *personal communication*). The largest is curved, stout, and often blunt, and shares a raised base with another large sensory hair (ass. s. h.). The second spine is more basal and lies adjacent to a patch of several very short, densely-arranged bristles.

The palp of the maxilla is one-segmented (Fig. 31, 32). The length of the palp in relation to the maxillary lobes varies among species. The palp bears six to ten sensory hairs over its pigmented surface and four to six sensory papillae (s. pap.) on its apex. There is a patch of five to ten sensory hairs at the base of the palp. These numbers of sensory hairs and papillae vary with species. On the ventral surface of the cardo there is a patch of 20 to 25 fine, unpigmented bristles. *S. venustum* larvae have an additional patch of fine bristles basal to the maxillary lobe.

The maxilla has two muscles. The retractor, consisting of three bundles, inserts on the

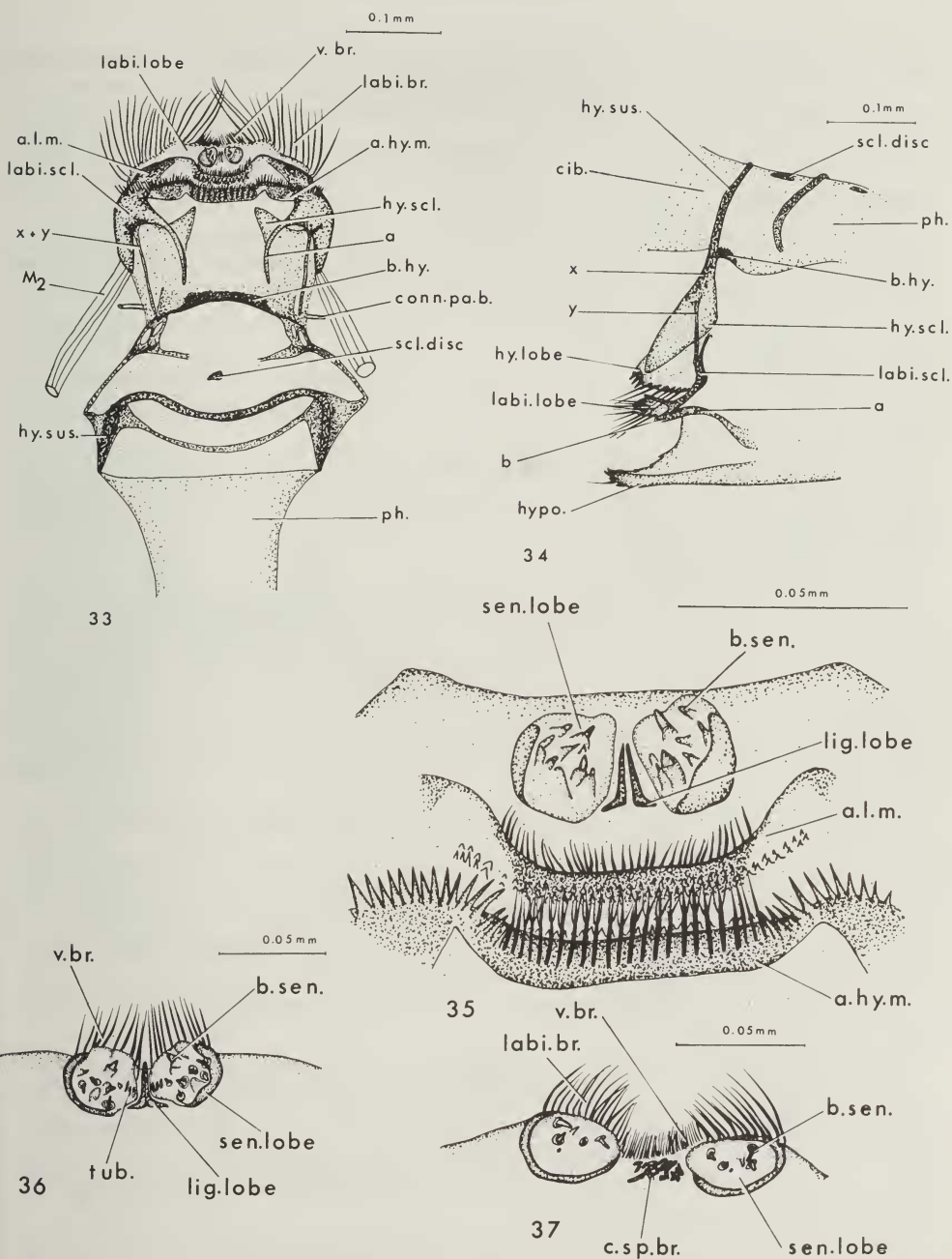


Fig. 33-37. Fig. 33-34: Labio-hypopharyngeal complex of *S. vittatum*. 33, dorsal view; 34, lateral view. Fig. 35-37: apex of labial lobe. 35. *S. vittatum*; 36, *C. dacotensis*; 37, *P. travisi*. a = bar 'a', a. hy. m. = anterior margin of the hypopharyngeal lobe, a. l. m. = anterior margin of the labial lobe, b = bar 'b', b. hy. = base of hypopharynx, b. sen. = basiconic sensilla, cib. = cibarium, conn. pa. b. = connection to postantennal buttress, c. sp. br. = conical spine-like bristles, hypo. = hypopharynx, hy. lobe = hypopharyngeal lobe, hy. scl. = hypopharyngeal sclerite, hy. sus. = hypopharyngeal suspensorium, labi. br. = labial brush, labi. lobe = labial lobe, labi. scl. = labial sclerite, lig. lobe = lobes of the ligula, M₂ = labial retractor muscle, ph. = pharynx, scl. disc = sclerotized disc, sen. lobe = sensory lobe, tub. = tubercles, v. br. = ventral brush, x = bar 'x', y = bar 'y'.

middle of the oral surface level with the base of the palp. It originates near the posterior border of the postgena, and moves the maxilla dorsomedially towards the cibarium. The extensor inserts basal to the retractor at the medioventral corner of the postgena, ventral to the retractor. It moves the maxilla ventromedially. The palp has no muscles (Craig, 1968).

The maxilla of the first instar of *S. vittatum* is fully developed. The bristles are smaller and fewer; the palp has two or three sensory hairs.

Labio-hypopharyngeal complex. — The labium and the hypopharynx are considered together because they form a complex unit and because the homologies of the labium are under dispute (Crosskey, 1960; Craig, 1969). The labio-hypopharyngeal complex (l-h., Fig. 33, 34) fills the ventromedial part of the mouth area. The complex is broadly semicircular and is in two main parts. The dorsal part, called here the hypopharyngeal lobe (hy. lobe), lies directly over the ventral labial lobe (labi. lobe). Both lobes are weakly sclerotized but are strengthened by a complicated set of relatively strong sclerites. They are covered ventrally by the hypostomium.

The dorsal surface of the base of the hypopharynx is strongly sclerotized (b. hy.) and marks the anterior of the cibarium. It is contoured to fit the labrum. At the lateral margin of this ridge the suspensorium is connected to the ventroposterior arm of the postantennal buttress by membranous cuticle (conn. pa. b.).

The anterior margin of the hypopharyngeal lobe has two rows of bristles (Fig. 35). The medial bristles of the posterior row are compound. The labial margin bears one row of short bristles and one row of blunt, paired teeth. In *C. dacotensis* and *S. venustum* larvae, the medial bristles are compound; in other species studied, they are simple. Grenier (1949) numbered the rows of hypopharyngeal and labial bristles; rows one to three are hypopharyngeal and row four is labial. His third row of the hypopharynx is probably the medial bristles of the anterior row which are longer than their fellows and point dorsally rather than anteriorly.

The labial lobe lies ventral to the labial margin and bears a brush on its ventroapical surface (labi. br.). The dorsoapical surface bears two prominent spherical sensory lobes (sen. lobe, Fig. 35 to 37). These bear a number of basiconic sensilla (b. sen.). *C. dacotensis* larvae have six on each lobe. *Simulium* species have six to eight and *Prosimulium* species have five. Some species have a trio of small tubercles (tub., Fig. 36) on the medial part of the sensory lobes. Rubtsov (1964) referred to them as *Höcker* (= tubercle). In *C. dacotensis* and *S. decorum* larvae they are crescent-shaped; in *S. venustum*, circular. *Prosimulium* and *S. vittatum* larvae lack them.

The lobes of the ligula (lig. lobes) are present medial to the sensory lobes (Fig. 35, 36). These are paired, slender, L-shaped, and strongly sclerotized. Ventral to the lobes of the ligula there is a small brush of short bristles (v. br., Fig. 36, 37). The *Prosimulium* species studied have a group of stout, spine-like bristles similar to those of the dorsum of the labrum (Fig. 37) in place of paired ligular lobes. These are immediately dorsal to the ventral brush and medial to the sensory lobes.

The silk (salivary) canal (sk. can.) is formed dorsally by the hypopharyngeal lobe and ventrally by the labial lobe. The paired salivary ducts pass anteriorly from the salivary glands along the midline of the head capsule and fuse to form the silk canal in the medioventral part of the head. The silk canal continues forward and expands laterally at the level of the first hypopharyngeal bar to join the corners of the labio-hypopharyngeal complex. The wall of the silk canal is reinforced by annular thickenings in its cuticular intima. The fusion of the salivary ducts begins ventrally, and the dorsal surface of the canal has a dorso-medial projection formed from the dorsal walls of the ducts for some distance anteriorly (Fig. 57). The silk thread emitted from the silk canal is dorsoventrally flattened and grooved

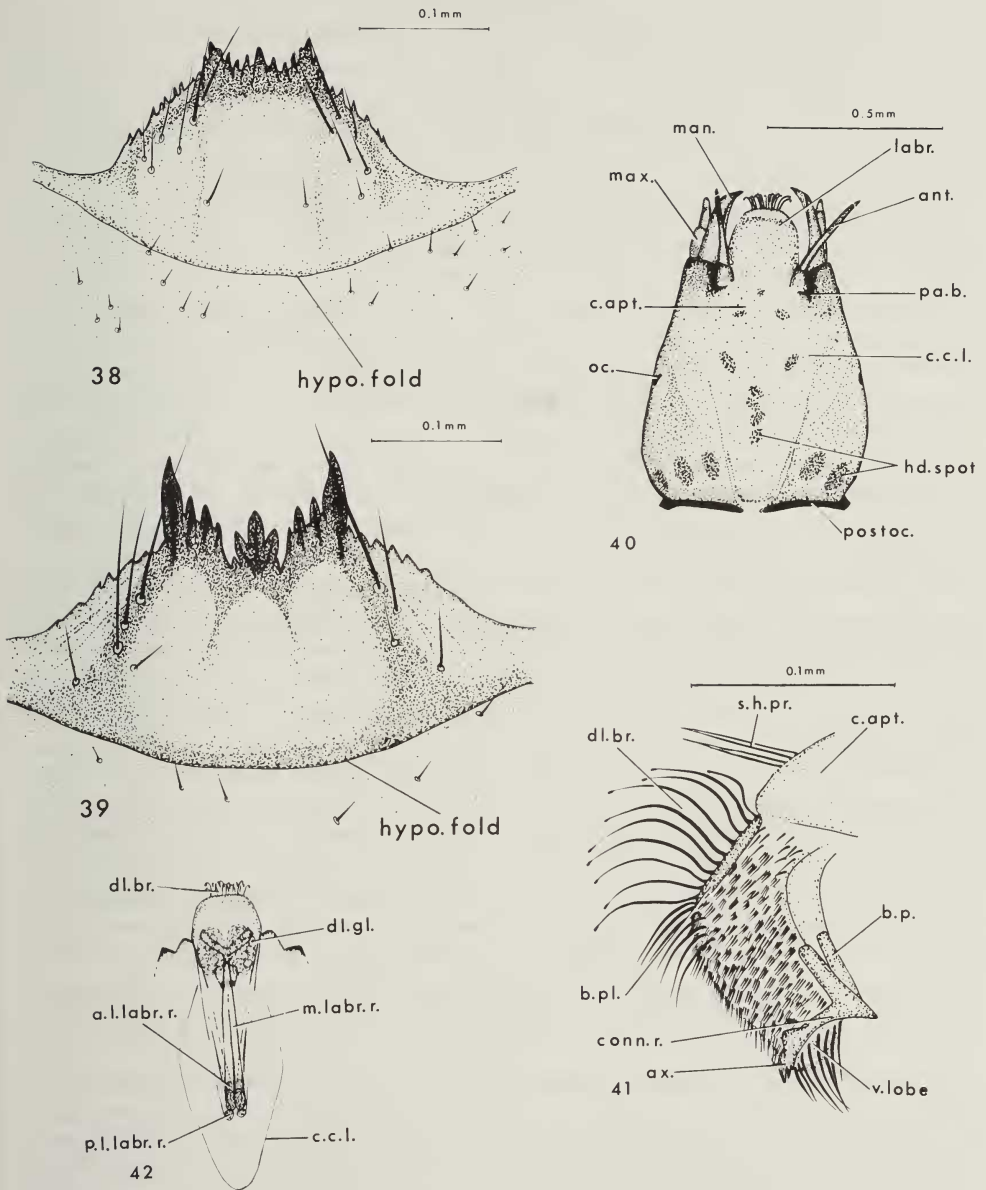


Fig. 38-42. 38, Hypostomium of *S. vittatum*. 39, hypostomium of *P. travisi*. 40, head capsule of *T. biclavata*. 41, labrum of *T. biclavata*, lateral view. 42, sketch of labral musculature of *T. biclavata*, not to scale. a. hy. m. = anterior margin of hypopharyngeal lobe, a. l. labr. r. = anterior lateral labral retractor muscle, a. l. m. = anterior margin of the labial lobe, ant. = antenna, ax. = apex of labral sclerite, b. p. = basal piece, b. pl. = basal plate, b. sen. = basiconic sensilla, c. apt. = cephalic apotome, c. c. l. = cephalic cleavage lines, conn. r. = connecting rod, c. sp. br. = conical spine-like bristles, d. gl. = dorsal gland, dl. br. = dorsal brush, hd. spot = head spot, hypo. fold = hypostomial fold, labr. = labrum, labi. br. = labial brush, lig. lobe = lobes of the ligula, sen. lobe = sensory lobe, man. = mandible, max. = maxilla, m. labr. r. = medial labral retractor muscle, oc. = ocellus, pa. b. = postantennal buttress, p. l. labr. r. = posterior labral retractor muscle, postoc. = postociput, s. h. pr. = pair of sensory hairs, tub. = tubercles, v. br. = ventral brush.

along the mid-dorsal line, either because of the dorsomedial projection of the canal or the paired salivary ducts.

The labio-hypopharyngeal complex has two pairs of muscles. The paired muscle of the 'press' of the silk canal, M_3 (after Grenier, 1949) stretches from the dorsolateral surface of the hypopharynx to the roof of the silk canal (Fig. 57). The second muscle, M_2 (after Grenier, 1949), inserts on the posterior tip of the labial sclerite (Fig. 33) and originates at the posterior margin of the postgenae adjacent to the postgenal cleft. It pulls the labial lobe posteriorly.

There is a pair of ventral glands (v. gl., Fig. 57), histologically resembling the dorsal glands, at the corners of the labio-hypopharyngeal complex adjacent to the labial sclerite. The glands have no apparent opening.

Hypostomium. — The hypostomium is a double-walled triangular plate of strong cuticle. The margin of the inner wall is distinguished by the hypostomial fold (hypo. fold, Fig. 38, 39) (Wood, 1963). The hypostomium is concave, forming a sheath for the labio-hypopharyngeal complex. The midline is invaginated dorsally near the apex and forms a ridge between the two sensory lobes of the labium.

The anterior margin of the hypostomium is strongly sclerotized and bears numerous teeth which have patterns of specific diagnostic value (Fig. 38, 39). The taxonomically important characters are the number of teeth, their relative sizes, and their simple or compound nature.

The hypostomium bears two rows of sensory hairs, one parallel to each of the lateral, dentate margins. The apical hairs are longer than the basal ones. The numbers of hairs vary with larval instar and with species: *C. dacotensis* larvae have one to five; *S. decorum* larvae, two to five; *S. venustum* larvae, two to six; *S. vittatum* larvae, two to nine; *Prosimulium* larvae, three to four. The number increases with instar. Ventral to the hypostomial fold there is a number of randomly-arranged sensory hairs.

Cibarium. — The anterior margin of the cibarium (cib., Fig. 34) is marked by the sclerotized base of the hypopharynx. Anterior to this lies the epipharynx dorsally and the hypopharynx ventrally (Fig. 56). The walls of the cibarium are reinforced by the hypopharyngeal suspensorium (hy. sus., Fig. 33, 34). Midway along its length, the cibarial wall thickens and becomes corrugated. There is a depression in the ventral surface. This region of the cibarium is provided with fine bristles which are continuous with groups of small bristles present on the epipharynx. The bristles on the wall of the ventral depression are longer than those of the dorsal surface. In the midline the bristles are stout, blunt, and conical, resembling those of the spindle-shaped patch on the labrum. Both epipharyngeal and cibarial bristles are directed posteriorly.

The cibarium has two pairs of muscles. Both are dilators. The anterior pair inserts medially on a sclerotized disc on the dorsal wall of the cibarium in between the two rings of the hypopharyngeal suspensorium (disc, Fig. 33, 34). It originates on the dorsal part of the cephalic apotome lateral to the labral retractor, at the anterolateral head spots. The second smaller pair of muscles inserts on a smaller sclerotized disc in the midline of the dorsal wall of the cibarium, posterior to that of the anterior muscles. This originates on the cephalic apotome just posterior and adjacent to the anterior pair of muscles.

Comparison with a non-filtering species

The head capsule of the larva of *Twinnia biclavata* Shewell is more tapered anteriorly (Fig. 40) than that of the other species of blackflies studied. The cephalic cleavage lines converge both anteriorly and posteriorly and the ends of the postocciput meet dorsally in the midline. The ventral wall of the head capsule is almost complete. The postgenal cleft

is very shallow and the postgenal bridge is complete. The antennae have four articles. There are two sensory papillae distally on the second article.

Labrum. — The labrum of larvae of *Twinnia biclavata* is joined to the cephalic apotome by a membranous area (mem. ar.). The anterior margin of the cephalic apotome is straight rather than curved as in filtering species. The arrangement of the labral bristles differs greatly from that of filtering species studied. There is a well-developed dorsal brush (dl. br.) of simple bristles which are dark, blunt, and curved at their apices (Fig. 41, 43). They differ from those of the larvae of *Twinnia tibblesi* Stone and Jamnback which are pectinate (Davies, 1965). The base of the brush is reinforced by a sclerotized plate, termed the basal plate (b. pl.) by Davies (1965). Immediately ventral to this brush there is a group of thinner, simple bristles. These are shorter than those of the dorsal brush but longer than the rest of labral bristles. They curve ventrally. The lateral and apical areas of the labrum are covered with smaller simple bristles in groups of two to five. All of them are directed medially. The ventral lobe (v. lobe) of the labrum is supported by the connecting rod (conn. r.) of the labral sclerite (labr. scl.). It bears simple bristles which are not so well developed as the compound bristles of the filtering species studied.

The labral sclerite is similar to those of the larvae of the other species studied. The apex (ax.) has 10 to 12 teeth along the anterior margin (Fig. 43). These are longer than the labral teeth of the filtering species studied. The basal piece of the labral sclerite is orientated at right angles to the connecting rod (Fig. 41). It supports the ventral surface of the labrum and epipharynx. The medial pair of sensory hairs on the dorsum of the labrum is well developed. These are immediately posterior to the dorsal brush and not on the cephalic apotome as in other species. There is a scattering of small sensory hairs on the cephalic apotome. The dorsal gland fills the cavity of the labrum. Histologically it is the same as that found in other species but it is composed of fewer cells.

The labrum of *T. biclavata* has two pairs of labral retractor muscles (Fig. 42). The medial pair is homologous with the medial retractor muscle of filtering species. It originates on the midline of the posterior half of the cephalic apotome and inserts on the ventral surface of the labrum immediately posterior to the connecting rod of the labral sclerite. The lateral pair is smaller and each muscle consists of two bundles. These originate respectively anterior and posterior to the medial retractor muscles. The origins of the lateral retractors are marked externally by the median head spots. These lateral muscles pass between the two lobes of the dorsal gland and insert dorsal to the medial retractors at the lateroposterior margins of the labrum. The lateral retractors differ from those described by Davies (1965) in *Twinnia tibblesi* larvae in that they do not insert on the curved rod (c. r.) which articulates with the basal plate of the dorsal brush (see below). Furthermore, the lateral retractors of *T. tibblesi* larvae consist only of one bundle each and this originates posterior to the medial retractors. The insertion of the lateral muscle on the curved rods in *T. tibblesi* indicates that this muscle is homologous to the cephalic fan retractors (Davies, 1965). This suggests here that the two bundles of the lateral muscles and their individual origins in *T. biclavata* possibly foreshadow the complex origin of the three-bundled cephalic fan retractors.

The pair of curved rods present in *T. tibblesi* larvae and *Gymnops* sp. larvae (Davies, 1965) is present in *T. biclavata* larvae. The rods lie lateral to the basal plate and appear to articulate with it. They do not form an X-shaped complex as illustrated by Davies for *T. tibblesi* (his Fig. 47, 1965). The curved rods (c. r., Fig. 43, 44) are immediately anterior to the bare knobs found in filtering species adjacent to the cephalic fan stem (Fig. 1, 3). They are in the same relative position as the cephalic fan stem sclerites in filtering species. Davies points out that the orientation of the rods is nearly at right angles to the cephalic fan

stem sclerites. He rejects the theory that the rods or the cephalic fans are tormal, and suggests that the rods are homologous with the cephalic fan sclerites. Wood (1963) considered the curved rods, the sclerites of *Gymnopsais* larvae, and the cephalic fan sclerites all tormal.

Mandible. — The mandible of *T. biclavata* is shorter and stouter than that of filtering species (Fig. 45). The angle of articulation is more parallel to the plane of the longitudinal axis of the body than it is in other species. Therefore, according to Cook's theory (Cook, 1949), *T. biclavata* larvae are more advanced in this respect than filtering forms. The mandible articulates with the postantennal buttress. This is less well developed than it is in the other species studied, having only three arms, one passing on each side of the antennal base and one forming the dorsal mandibular articulation. The fourth arm is represented only by a pocket of rigid cuticle formed by the invagination and thickening of the margin of the head capsule. A projection of the medial side of the mandible articulates with this pocket.

The retractor muscle of the mandible is very well developed. It is composed of four large bundles inserting on a large, sclerotized apodeme located on the oral surface of the mandible. The bundles originate laterally on the posterolateral parts of the postgenae, ventral to the extensor muscle. The ventral bundles originate adjacent to the postgenal cleft; the dorsal, immediately lateral to the labral muscles. The extensor muscle consists of four smaller bundles which insert on a shorter, non-sclerotized apodeme on the adoral side. They originate lateral and dorsal to the retractor muscle. Both these muscles are larger than those of filtering species.

The arrangement of teeth on the mandible differs from that of the other species studied (Fig. 46). There are 10 to 12 teeth arranged in a curved line along the apex. The dorsalmost teeth are largest with the size decreasing towards the base of the mandible. There is an extra basal tooth between the fourth and fifth, or fifth and sixth teeth from the apex. The apices of the teeth are occasionally broken off, presumably during feeding. There are two minute, apically-directed marginal teeth. Three apical spines are present behind some small spines near the base of the marginal teeth.

The brushes of the mandibles are less well developed than those of filtering species (Fig. 45). There are no covering, apical or second external brushes and the first external brush is shorter and composed of small bristles. In filtering species, these four brushes comb the fans. The external lobe which separates the two apical brushes from the mandibular teeth is lacking, as is the inner brush. The bristles of the middle, small basal, and large basal brushes are fewer and finer than those of the filtering species. Only some of the bristles of the large basal brush are compound. The middle lobe is present.

The mandibles have fewer sensory hairs. There is one large sensory hair at the base of the mid-dorsal line and a second about half way up the mid-dorsal line. Some more sensory hairs are present on the oral and adoral surfaces. The pair of sensory hairs with a common base is immediately behind the base of the first external brush and both are smaller than those found in other species.

Maxilla. — The maxilla of *T. biclavata* larvae is similar in shape to that of other species but the maxillary lobe is more tapered at the apex (Fig. 47, 48). The arrangement of brushes is different. There is only one oral brush (or. br.) which has bristles arranged in rows. This is adjacent to the lacinia. The lacinia has numerous patches of very small bristles arranged in groups of two to five, however, it does not have the bristles on its distal border. Apical to the oral brush, the middle brush (m. br.) has long, orally-directed bristles. The apical brush (a. br.), found on both oral and adoral surfaces, has large bristles directed apically. Another large brush, the second apical brush (2 a. br.), lies ventral to the apical brush on the adoral side; this brush is not found in the filtering species. The bristles of the two apical brushes are thick and curved at their apices. The rest of the dorsal surface is bare.

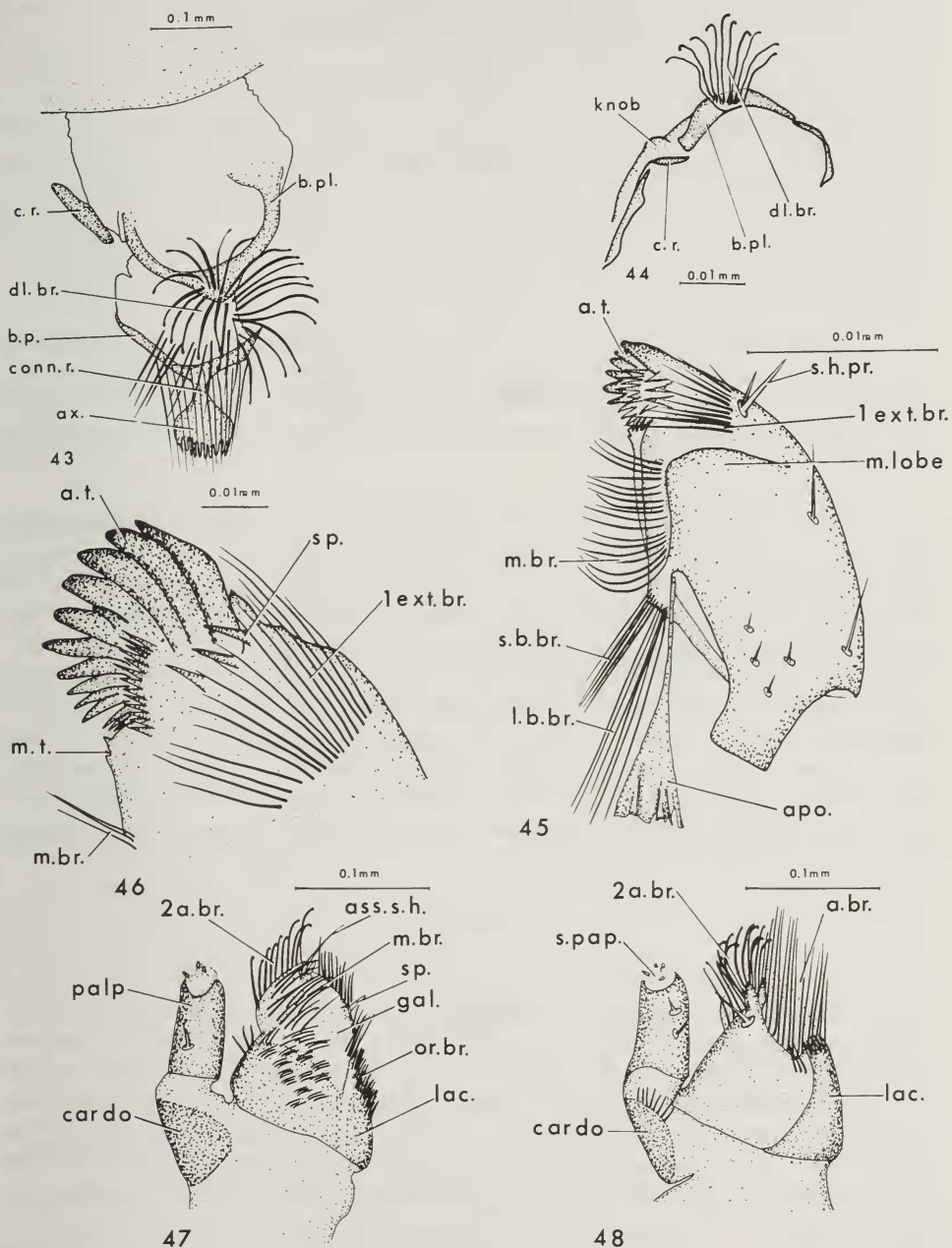


Fig. 43-48. *T. biclavata*. 43, labrum, dorsal view; 44, curved rods; 45, mandible, lateral view; 46, detail of apex of mandible; 47, right maxilla, ventral view; 48, left maxilla, dorsal view. a. br. = apical brush, 2 a. br. = second apical brush, apo. = apodeme, ass. s. h. = associated sensory hair, a. t. = apical teeth, ax. = apex of labral sclerite, b. pl. = basal plate, c. r. = curved rod, conn. r. = connecting rod, dl. br. = dorsal brush, 1 ext. br. = first external brush, gal. = galea, l. b. br. = large basal brush, lac. = lacinia, m. br. = middle brush, m. lobe = middle lobe, m. t. = marginal teeth, or. br. = oral brush, s. b. br. = small basal brush, s. h. pr. = pair of sensory hairs, sp = spine, s. pap. = sensory papillae.

There is no lobulate area.

The maxillary lobe has two spines (sp.). These are much shorter than those of the other species studied. Neither spine has a raised base; the apical spine, however, does have an associated sensory hair (ass. s. h.). On the bare adoral surface there are two sensory hairs, one apical and one basal.

Both muscles of the maxilla originate anterolateral to the mandibular muscles and have two bundles. The smaller bundle of the retractor originates just below the ocelli and dorsal to the larger bundle. The extensor muscle originates on the posterior region of the postgena.

The maxillary palp is the same length as the maxillary lobe. It has three to four small sensory hairs scattered over the pigmented surface and three to four sensory papillae in the centre of the apex. There are two sensory hairs on the adoral side of the palp base. As in other species, a patch of the large bristles is present at the base of the palp.

Labio-hypopharyngeal complex. — The labio-hypopharyngeal complex of *T. biclavata* is similar to that of the other species studied, but the sclerotized framework is less well developed. The connection between the hypopharyngeal sclerite and the hypopharyngeal suspensorium is membranous. Only one ring of the hypopharyngeal suspensorium surrounds the gut.

The hypopharyngeal lobe has two anterior rows of bristles and spines. The dorsal one consists of short spines rather than bristles. The labial margin is not so well developed as that of the other species studied. The ventral brush has uniform bristles of equal length. They are distinct from those of the labial brush but not separated from them (Fig. 49).

The two sensory lobes each bear three sensory papillae but no trio of tubercles. The lobes of the ligulae are paired but they curve laterally at the apex.

The musculature of the labio-hypopharyngeal complex, the ventral glands and the salivary ducts are all similar to those found in the filtering species.

Hypostomium and cibarium. — The hypostomium of *T. biclavata* larvae (Fig. 50) has compound teeth. There are three sensory hairs per row and there is one mediobasal pair.

The cibarium of *T. biclavata* larvae does not differ from that of the other species. Two pairs of dorsal cibarial muscles are present but these are smaller than those of filtering species and do not insert on sclerotized discs.

FEEDING

Feeding behaviour

Blackfly larvae attach themselves to a silken pad on the substratum, usually within 8 to 10 centimeters of the surface of the water, with their posterior circlet of hooks. They attach dorsal side down and rotate their body some 90 to 180 degrees to the left or right between the fourth and fifth segments. They are orientated with respect to the current so that the head is downstream from the abdomen and is held so that the fans face the current. The angle between the substratum and the body is not actively maintained but varies with the current. Hocking and Pickering (1954) described the pattern of larval attachments in streams. Fortner (1937) described the feeding stance in detail.

Both primary and secondary fans catch particles. The particles held in the centre of the primary fans may be retained there through three or four flicks (retractions and extensions) of the fans, or they may be swept out of the fan before it closes. Loss of particles either from the fans or the mouthparts is frequent. Larvae may not flick their fans immediately on catching a particle. They may flick them without having caught any particles.

The frequency of flicking is irregular. A larva extends its fans for several seconds and then flicks them continually for several seconds. The fans are generally flicked alternately, one of

the pair retracting and extending, and then the other. The duration of periods of flicking and non-flicking varies greatly. The frequency of flicking does not vary either between late instars and young larvae or between larvae with full guts and larvae with empty guts.

Larvae retain food in the cibarium until a bolus is formed and swallowed. Some selection of the particles with respect to size occurs during the transfer of particles from fans to mandibles. Larvae will retract fans which have trapped particles of 800 microns in diameter, larger than the mouth orifice. These are passed to the mandibles and maxillae but are not ingested. Occasionally, however, large sephadex particles with diameters slightly larger than that of the intima were found in the gut, compressed into a cylindrical shape by the walls of the gut. These particles progressed through the gut and did not appear to harm the larvae.

The first instar larvae of *S. vittatum* filter with their cephalic fans. They are able to feed while suspended from the surface film of still water. They are very active. Some had only yolk in their guts; others had small pieces of charcoal, sephadex, and organic matter.

No cannibalism was observed among laboratory populations. However, three fights were observed. In all fights a larger larva attacked a smaller one. Larvae were never injured. One fight ended when the smaller larva moved out of reach of the larger one whereupon both larvae resumed feeding.

Movements of mouthparts of filtering larvae

Food collection. — A blackfly larva gathers food both by filtering particulate matter from the water with its cephalic fans, and by scraping organic material off the substratum. The first mechanism is the commoner. Particles caught in the cephalic fans are transferred to the cibarium when the fans are retracted and cleaned. The fans are believed to open by an increase in pressure of the body fluids in the fan stem (Grenier, 1949; Wood, 1963). This is probably the same mechanism as that described by Chaudonneret (1963) for the labrum. The opening of the fans of living or preserved larvae can be achieved by squeezing the thorax or cervical region of the larvae (Wood, 1963).

The primary fans open with the ventral movement of the apex of Sc_1 and the lateral movement of the ventral lobes of the cephalic fan stem (Fig. 53, 54). Infrequently, when the fan expands the primary rays are hooked over each other. When this occurs the larva immediately flicks the fan and the rays become unhooked. Fortner (1937) stated incorrectly that the closing mechanism of the cephalic fans prevented the rays from becoming entangled with each other. The secondary fan unfolds in a spiral movement. The medial fan moves laterally (with respect to the fan stem) with the movement of the medial lobe.

The fans are closed by the contraction of the cephalic fan retractor muscle. The fan stem sclerite Sc_1 moves posterobasally and its apex moves dorsally. This movement is combined with the dorsal and medial (with respect to the fan stem) movement of Sc_m (Fig. 53, 54). The apices of the ventral lobes of the stem move together (Fig. 53). The primary fan closes in response to the downward movement of Sc_1 as well as to the increased curvature of the primary fan base due to the movement of the ventral lobes. The rays move together one after another like the struts of a venetian blind being raised. The inner rays move first. As the expanded bases of the rays act as buffers (Fig. 6), the closing of the fan is smooth and regular. The secondary fan closes in a similar way.

The rays of the medial fan do not diverge from one another at any time. This fan closes when the medial lobe moves medially prior to the closing of the primary fan. Both secondary and medial fans lie underneath the primary fan when the latter is retracted (Fig. 54).

The stem of the cephalic fan moves medially and orally when the fan is retracted. The sclerites Sc_b and P_b act as fulcrums. The cephalic fans have two positions of retraction. The more frequently occurring is the retraction for cleaning. If the larvae are disturbed, the fans

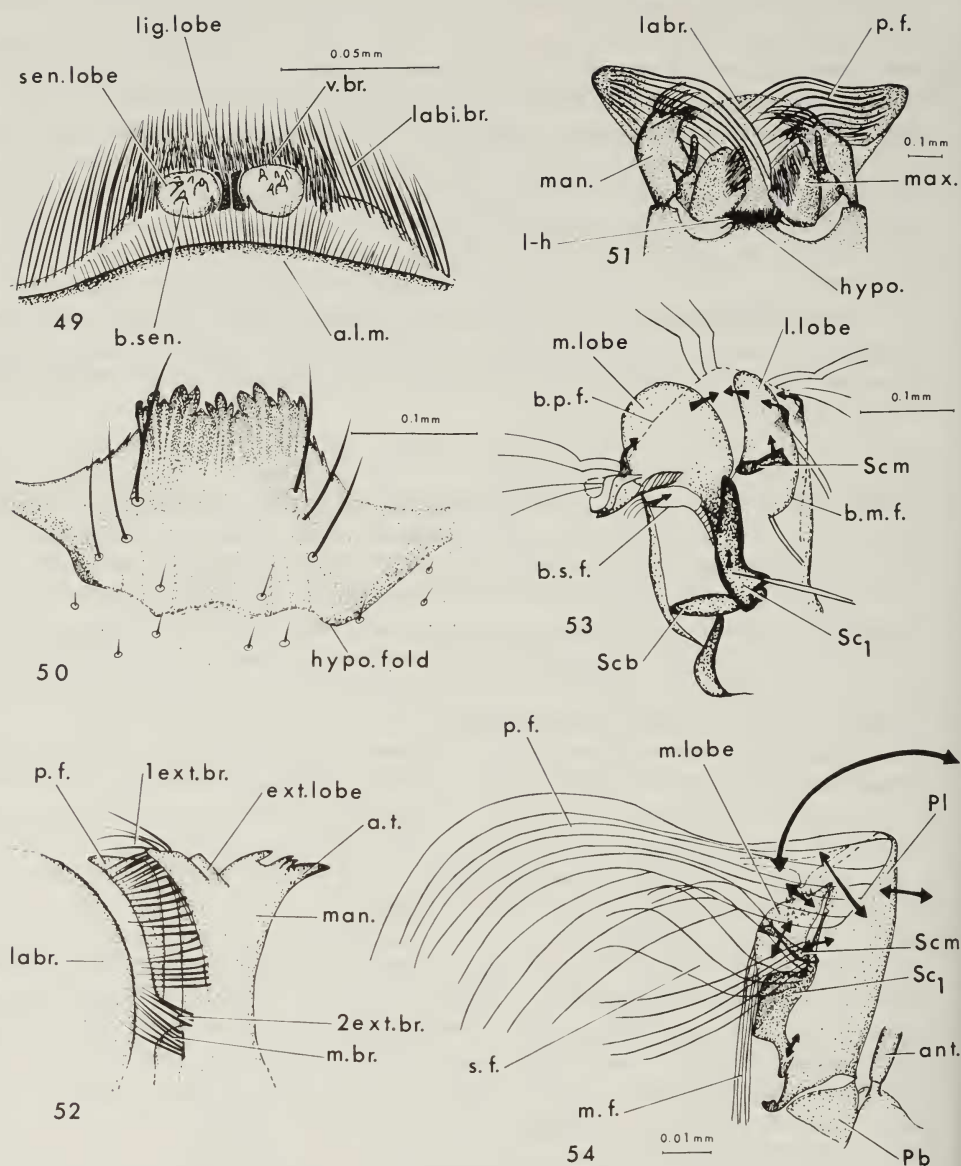


Fig. 49-54. Fig. 49-50: *T. biclavata*. 49, apex of labial lobe; 50, hypostomium. Fig. 51-54: *S. vittatum*. 51, position of retracted fans; 52, sketch of retracted fans when rays are combed, not to scale; 53-54, movements of elements of fan stem; 53, ventral view; 54, lateral view. a. l. m. = anterior margin of the labial lobe, ant. = antenna, a. t. = apical teeth, b. m. f. = base of medial fan, b. p. f. = base of primary fan, b. sen. = basiconic sensilla, b. s. f. = base of secondary fan, 1 ext. br. = first external brush, 2 ext. br. = second external brush, ext. lobe = external lobe, hypo. = hypostomium, hypo. fold = hypostomial fold, labr. = labrum, labi. br. = labial brush, lig. lobe = lobe of the ligula, l-h. = labio-hypopharyngeal complex, l. lobe = lateral lobe, man. = mandible, max. = maxilla, m. f. = medial fan, m. lobe = middle lobe, p. f. = primary fan, sen. lobe = sensory lobe, s. f. = secondary fan, v. br. = ventral brush.

are retracted further into the mouth orifice. In the latter case the mandibles and maxillae are also retracted.

The labrum, mandible, and maxilla have a simple movement of retraction and extension. All three appendages may twitch rapidly, a motion best described as 'shivering'. The mandibles usually move simultaneously. Although they can move independently they do so rarely. They are extended when the cephalic fans are retracted and they then retract to clean the closed fan. During periods of intensive feeding, the mandibles move in conjunction with the labrum and maxillae, being extended and retracted at the same time. Infrequently the labrum and maxillae are retracted while the mandibles are extended.

On several occasions larvae in the laboratory caught asymmetrical particles. These were transferred to the cibarium by the mandibles and maxillae and ingested. Although no special manipulatory movements were seen, the particles entered the cibarium with their longitudinal axes parallel to that of the body. The shape of the cibarium coupled with the movements of the mouthparts and the folding of the fans appear to orientate particles.

The second form of feeding is that of scraping material off the substratum. The head is held more or less at right angles to the substratum. The whole body is used, at times twisting in a complete circle to wrench material free. The labrum, mandibles and maxillae sometimes 'shiver' while the larvae feed off the substratum.

The mandibular teeth scrape the surface. The orientation of the apical teeth is suited for this function. The external lobe of the mandible prevents the apical brushes from scraping although these brushes may collect superficial material scraped free by the mandibular teeth. The bristles of the labrum, especially those of the ventral lobe, also collect material. The labral teeth are too well covered by bristles to be useful in substratum feeding. The position of the hypostomial teeth, slightly more dorsal than the rest of the hypostomium, and their covering of sensory hairs suggests that the hypostomium is not used in scraping.

Combing. — The transfer of particles from the fans to the cibarium occurs when the fans retract. The fans are combed by the mandibles and labrum (Fig. 51). The inner surface of the mandible is contoured to fit the curve of the folded fan and the labrum similarly fits the curve of the labral surface of the fan (Fig. 52). The folded fan passes underneath the covering and first external brushes of the mandible and above the middle lobe of the mandible. These brushes comb the convex surface of the folded fan. The second external brush passes beneath the fan. The mandibles do not clean the labral surface of the fans as described by Fortner (1937). The mandibles are very active during feeding and comb the fans several times during one retraction of the fans. Both mandibles and labrum retract while the fans are extended, combing each other free of particles. When the fans are held extended for long periods, the mandibles are also extended.

The fans are well adapted for filtering; the mandibles are well adapted for combing the fans. The development of the labral bristles and mandibular brushes, the shape of the labrum and mandibles as well as the development of the complex fan are major adaptations for filtering. Furthermore both labrum and mandible are also capable of scraping the substratum. However, these are not as efficient scraping appendages as those of the non-filtering species. The bristles of the labrum and the plane of movement, teeth, and musculature of the mandibles of *T. biclavata* larvae are better adapted for scraping. The mandibles of *T. biclavata* larvae are not adapted for combing fans.

The maxillae are continually active during feeding. Their role is less well defined than that of the other mouthparts. The arrangement of brushes differs between filtering species and non-filtering species yet both types of larvae have maxillae well provided with brushes. This suggests that the maxillae do not assist in filtering or combing but have a similar function in all species.

The frequent retractions of the labrum, mandibles and maxillae may contribute to the ingestion of food by pushing particles into the mouth. The ventral compound bristles of the labrum and the basal brushes of the mandible enter the pharynx. When the primary rays of the cephalic fan enter the pharynx, these bristles may comb the apices of the rays; they are not in a position to comb the rays outside of the cibarium. However, the large basal and middle brushes may act as guides for the rays or food particles; they may keep the epipharynx and the bases of the fan stem, mandibles, and maxillae free of particulate matter. The inner brush of the mandibles protects the mandibular cleft; it has no role in combing.

During feeding the proleg is held close to the body with its apex just below the hypostomial teeth. This position contributes to the streamlining of the body. The proleg is often brought close to the mouthparts and cleaned of debris or silk.

Silk secretion. — Blackfly larvae secrete silk very rapidly. Within a few seconds a larva can select a new site of attachment, produce a silk strand, apply it to the substratum and hook into it with its posterior circlet of hooks. Throughout the process the fans are extended, and held out of the way of the sticky secretion. The mandibles, maxillae and labio-hypopharyngeal complex are in constant and rapid motion. The labio-hypopharyngeal complex moves anteroposteriorly. The mechanism by which the silk is brought anteriorly and out of the canal is not clear. The M_3 muscle of the labio-hypopharyngeal complex dilates the silk canal and silk may be sucked forward by the increase in diameter of the canal. The simultaneous action of the retractors of the labio-hypopharyngeal complex may help this movement. The labio-hypopharyngeal complex acts as a press, and the activity of the M_2 and M_3 muscles may contribute to the dorsoventral flattening of the silk strand. The constant motion of the mouthparts and the body may also aid in the anterior movement of silk. The head is repeatedly extended and retracted; it arches upwards and stretches forwards. The proleg hooks onto the silk strand and draws it down from the silk canal. The mouthparts are then applied to the substratum and the silk, which is very sticky, adheres readily. Either the proleg or the posterior circlet of hooks then hooks into the pad. The hypostomial teeth sever the strand (Wood, 1963). Since the teeth do not move, the strand must be drawn across them by the movement of the labio-hypopharyngeal complex. The larva may maintain its position by using the unsevered strand until it hooks on with its proleg or posterior circlet of hooks.

The silk thread is very strong. Large larvae can be lifted out of water on the end of a six inch strand. The strand is used as a safety line by which floating larvae catch onto projections of the substratum. Larvae climb up their strands using their prolegs and mouthparts. Hora (1930) suggested that larvae use the stout bristles of the labrum to climb along the silk strand, however, the labral bristles of the species studied are not structurally suited for this task. It is probable that the mandibles and maxillae are used. Feeding larvae have no anchor line and rely on the posterior circlets of hooks for attaching onto the substratum.

The pupal case is made of silk. Peterson (1956), Hinton (1958b), Burton (1966) and others have described the spinning of the cocoon. The fans are retracted and the mandibles rarely move during cocoon formation. A pharate pupa spins the cocoon which is constructed of a leathery, coloured silk probably of a different composition from that of larval silk.

Mouthpart movements of a non-filtering larva

Larvae of *Twinnia biclavata* were observed feeding in still and flowing water. The larvae graze the substratum. Attached by their prolegs, the larvae feed off the substratum in front and to the side. The mouthparts are very active; the labrum and maxillae retract while the mandibles extend. The labrum moves so that its dorsal brush scrapes the substratum. The

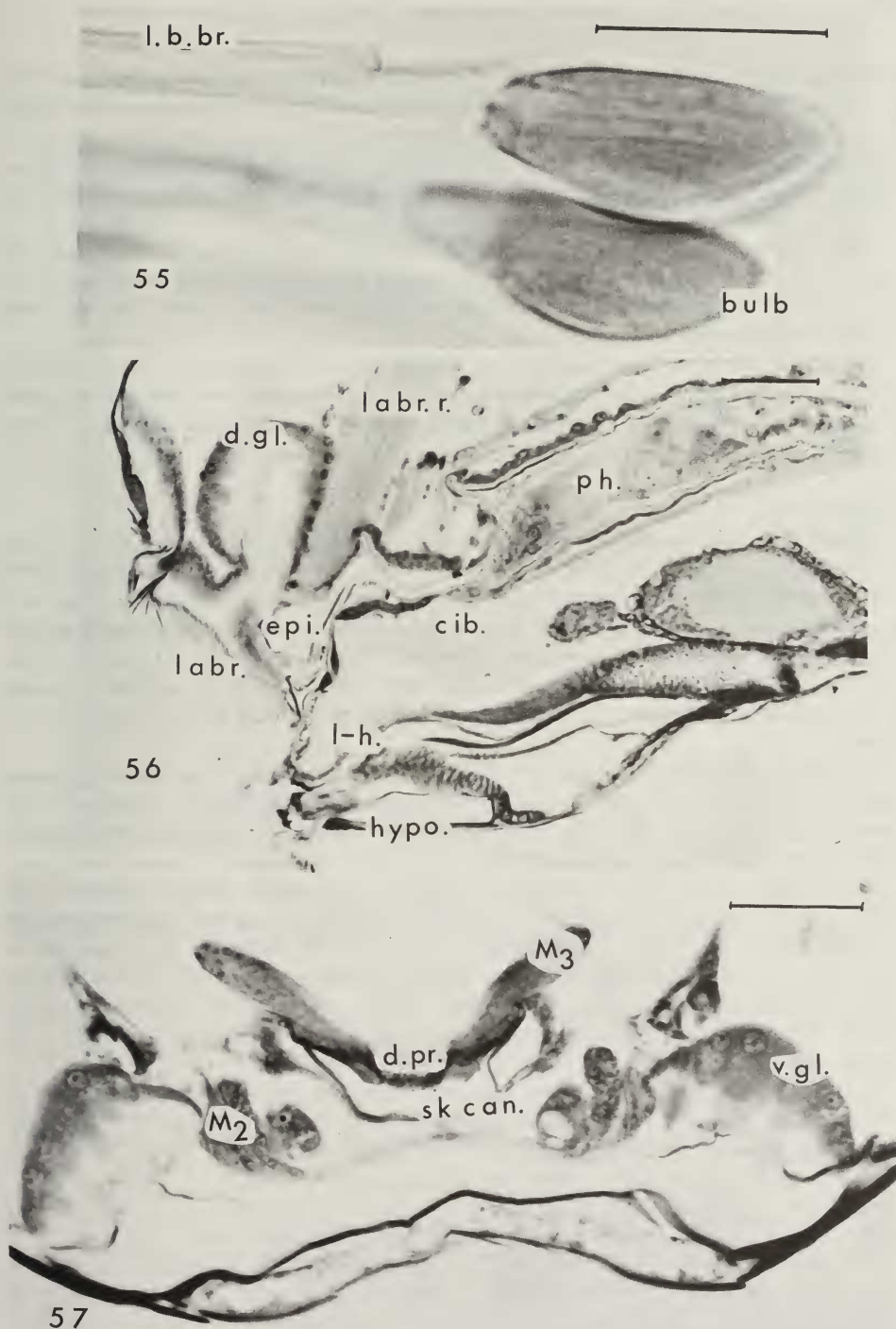


Fig. 55-57. 55, Photomicrograph of bulbs found on the large basal bristles of the mandibles of *S. vittatum*; 56, photomicrograph of a sagittal section of the head capsule of a blackfly larva; 57, photomicrograph of a transverse section of the ventral half of the head capsule of a blackfly larva. Scale for Fig. 55-57 is 5 microns. cib. = cibarium, d. gl. = dorsal gland, d. pr. = dorsal projection, epi. = epipharynx, hypo. = hypopharynx, labr. = labrum, labr. r. = labral retractor muscle, l. b. br. = large basal bristle, l-h. = labio-hypopharyngeal complex, M₂ = labial retractor muscle, M₃ = muscle of the press, ph. = pharynx, sk can. = silk canal, v. gl. = ventral gland.

mandibles move nearly parallel to the labrum, their teeth scraping the substratum. The brushes of the maxillae are retracted and move dorsomedially with respect to the larval body. The mouthparts 'shiver' as do those of the filtering species. Periodically the larvae cease feeding and clean their mouthparts. Cleaning is achieved in the same manner as by filtering larvae, i.e., brushing the mouthparts against each other so that the mandibles clean the labrum and the maxillae and are in turn cleaned themselves.

Larvae were observed eating filaments of algae by progressively ingesting from one end along the filament. Larvae were also observed grazing the surface of strands of algae. They pick at the algae by grasping filaments between their mandibles and slowly moving their mouthparts along them.

Silk is secreted in the same manner as in filtering species. Having cleaned the substratum around themselves of food, larvae progress forwards or sideways to a new site. Larvae appear to search for a new site by raising their heads up from the substratum and waving them around.

Ingestion

Blackfly larvae are unselective with respect to the composition of their food. Gut contents of larvae collected from the field consist of leaf litter, spores, pollen, algae, pieces of plant stems and unrecognizable organic debris. Fragments of insect cuticle are the only recognizable animal matter present. These include pieces of blackfly larvae, pupal respiratory filaments, head capsule parts, and mandibles. The gut contents of larvae reared in the laboratory also had a large proportion of charcoal and, after the addition of yeast to the rearing jars, clumps of yeast. Silt and sand form the bulk of the inorganic components.

The organic contents of the gut are not always digested. There is no visible difference between contents in the foregut and rectum. Naumann (1924) stated that utilization of the ingested algae was slight. Maciolek and Tunzi (1968) stated that blackfly larvae digested diatoms but hardly affected organic detritus.

Measurements were made of 200 particles ingested by blackfly larvae collected in the field. These particles were among the largest the larvae ingested. The two largest dimensions of the particles were measured. Sizes ranged from 0.5 to 300 microns in length and 0.5 to 120 microns in width. Most particles were 20 to 100 microns long and 10 to 60 microns wide. Fragments of insect cuticle, which were flexible and may have been folded during feeding, were the largest particles ingested. The biggest of these were 500 x 160 x 120 microns, 440 x 120 x 40 microns, and 320 x 120 x 60 microns.

The smallest gut particles measured were 0.5 microns in diameter but smaller particles were abundant. Due to the nature of the organic debris which adheres to the microtrichia of the primary rays and which is transferred to the gut when the fan is cleaned, it was not possible to get accurate measurements of the smaller particles.

Diameters of particles ingested by first instars ranged from 0.5 to 4 microns. Second instars ingested particles with a maximum diameter of 8.5 microns.

The frequency distributions, expressed as percentages of sephadex beads ingested by larvae in the laboratory are tabulated below (Table 6a and b). The difference between the size distribution of beads available to the larvae and those ingested by the larvae was tested for significance using the Chi-square test. The level of significance was set at $P = 0.05$ with P being the probability. Frequencies of categories less than five within a species size were lumped and Yates' correction was applied. The mass median diameter (MMD) for the frequency distributions of ingested beads was determined both by calculation (MMD_c) and graphically (MMD_g).

Table 6a. Percentage frequency distributions of sephadex beads ingested by larvae of three species of blackflies, Summer 1966.

Diameter (microns)	% of beads available	% frequency distribution <i>C. dacotensis</i>			<i>S. venustum</i>	<i>S. vittatum</i>
		small	medium	large	medium	medium
25	0.0	0	0.0	0.0	0.0	0
45	0.0	4	0.0	0.14	2.1	0
65	3.0	0	1.9	1.15	3.1	0
85	4.0	8	19.1	12.1	7.3	0
105	1.5	13	9.3	6.05	2.1	0
125	16.7	25	32.1	28.39	22.9	0
145	11.6	29	15.4	13.11	6.3	0
165	18.2	17	13.0	17.72	14.6	0
185	15.2	4	2.3	4.03	14.6	40
205	2.0		7.0	14.12	11.5	20
225	4.0			0.43	5.2	20
245	12.1			2.16	8.3	20
265	3.5			0.14	0.0	
285	2.0			0.43	2.1	
305	0.5					
325	2.0					
345	0.0					
365	2.0					
385	0.5					
405	0.5					
425	0.0					
445	0.5					
Total nos. of beads ingested		24	215	649	96	10
MMD _c *	204	136	128	146	162	187
MMD _g **	134	134	142	162	170	188
Nos. of larvae		5	19	11	10	2

Range of mean no. of beads per millilitre = 17 to 32.

* mass median diameter, calculated

** mass median diameter, determined graphically

Most of the *C. dacotensis* larvae and the medium larvae of *S. venustum* and *S. vittatum* were exposed only to sephadex G-200 (Table 6a) as that was the only type then available. No small larvae of *S. decorum* and *S. venustum* were collected live from the field or reared.

Without exception the frequency distribution of sizes of ingested beads differed significantly from that available to the larvae in each species in each age group. Large *C. dacotensis* larvae ingested a frequency distribution size different from that of medium *C. dacotensis* larvae. All interspecific comparisons show statistically significant differences in the size distribution of particles ingested. No other comparisons show differences significant at the 5% level.

Table 6b. Percentage frequency distributions of sephadex beads ingested by larvae of four species of blackflies, Summer 1967.

Diameter (microns)	% of beads available	% frequency distribution ingested							
		<i>C. dacotensis</i> large	<i>S. decorum</i> medium large		<i>S. venustum</i> medium large		<i>S. vittatum</i> small medium large		
25	27.76	42.6	76.2	63.30	54.8	41.6	85.9	56.58	47.04
45	22.96	42.9	16.2	18.75	16.4	18.6	10.9	26.40	25.99
65	11.3	8.8	4.3	4.15	11.3	11.5	2.2	5.32	8.12
85	6.81	2.1	1.0	2.56	9.6	12.9	0.0	3.78	4.27
105	4.29	1.8	1.3	2.10	4.5	9.3	1.1	2.24	4.36
125	3.81	0.6	0.3	2.16	0.6	3.9		1.57	2.99
145	4.05	0.3	0.3	0.08	2.3	1.4		1.67	2.96
165	3.89	0.6	0.0	1.25	0.0	0.4		1.09	1.70
185	3.49	0.0	0.0	0.91	0.0	0.4		0.45	0.96
205	3.25	0.3	0.3	1.59	0.0			0.54	0.84
225	2.32			1.02	0.6			0.10	0.45
245	1.76			0.68				0.19	0.09
265	1.32			0.40				0.00	0.06
285	0.68			0.11				0.03	0.06
305	0.64			0.17				0.03	0.09
325	0.64			0.00					0.03
345	0.48			0.06					
365	0.28								
385	0.20								
405	0.08								
Total nos. of beads ingested		329	303	1760	177	279	92	3121	3348
MMD _c * 177		276	74	146	93	57	45	97	109
MMD _g ** 195		245	85	155	107	81	51	115	118
Nos. of larvae		2	5	5	1	2	9	20	14

Range of mean no. of beads per millilitre = 17 to 32.

* mass median diameter, calculated
** mass median diameter, determined graphically

The individual Chi-square values indicate that the large larvae, especially *S. decorum* and *S. vittatum* larvae, tended to select particles of a diameter of 25 microns.

The maximum size of sephadex particle ingested by each group of larvae is tabulated below (Table 7).

The minimum size which could be ingested was not determined because the smallest sephadex bead was still large enough to be trapped by the fans. Measurements of gut contents of larvae collected in the field included sizes of about 0.5 microns, 50 times smaller than the smallest size of sephadex bead.

The gut contents of field collected *Twinnia biclavata* larvae consisted mostly of diatoms with filamentous algae, spores, plant fragments and unidentifiable debris comprising the remainder of the organic material. A mixture of silt and sand, the second most common material, was the only inorganic material present.

Table 7. Maximum diameters (in microns) ingested by four species of blackfly larvae at three stages of development.

Species	small	medium	large
<i>C. dacotensis</i>	105	305	325
<i>S. decorum</i>	—	205	345
<i>S. venustum</i>	—	285	185
<i>S. vittatum</i>	185	205	285

Largest particles found in the guts of preserved (in 90% ethanol) *T. biclavata* larvae were plant fragments which ranged in size from 150 to 210 microns in length and 4 to 20 microns in width. Largest diatoms ranged from 124 to 170 microns long and 28 to 110 microns wide. Most particles ranged from 20 to 40 microns long and two to four microns wide.

Based on the concentration of beads available, 17 to 32 beads/ml, and the number of beads ingested per minute, the rate of filtration of six medium and six large larvae of *S. vittatum* were calculated to be 0.4 to 1.7 ml/min and 0.2 to 1.3 ml/min respectively. Because so little is known about the feeding behaviour of blackfly larvae, and because conditions in the rearing jars varied, these values should only be considered approximations of filtration rates.

DISCUSSION

Morphology

Head capsule. — The larval head capsules of the four filtering species studied are structurally very similar. There are no anatomical differences to which variations in particle size selection can be attributed. The only major morphological differences were seen between filtering larvae and *Twinnia biclavata* larvae, which do not filter feed.

Measurement of the head capsules, cephalic fans and mandibles of *C. dacotensis* and the *Simulium* species show that the head capsules of all four species are approximately the same size. The size of the head capsule and appendages increases with the growth of the larvae. Slight differences are shown by *C. dacotensis* and *S. vittatum* larvae. The head capsules of the larvae of these two species are larger than those of *S. decorum* and *S. venustum* (Table 1). The fans of both large *C. dacotensis* and *S. vittatum* larvae are bigger than those of the larger larvae of the other species, however, medium and small larvae of *S. vittatum* have larger fans than larvae of the same groups of the other species. The mandibles of large larvae of all filtering species are the same size; medium and small larvae of *S. vittatum* have larger mandibles than larvae of the same age of other species.

The three patterns of pigmentation of the anterior margin of the cephalic apotome of *S. vittatum* larvae are a variation within the species. This is one of several intraspecific anatomical variations found among *S. vittatum* larvae.

Cephalic fans. — Because *Prosimulium* is considered to be a primitive genus of blackflies, the occurrence of four to six intermediate rays in the cephalic fan and the presence of fewer blades suggests that the cephalic fans once consisted of a single big fan which has evolved into three differentiated fans. The central, largest fan is the principal filtering organ. However, the role of the secondary fan has given rise to speculation. Some authors consider the secondary fan to increase the filtering area of the primary fan since the secondary rays extend lateroventrally to the primary fan. Others maintain that it prevents particles from

falling among the bases of the primary rays or into the ventral wall thereby hindering the closing of the fan. It does both. Particles are caught by the secondary fan and those observed were not caught to the side of the primary fan. Further, the secondary rays extend distal to the bases of the primary rays; the dense trichiation prevents particles from falling among the primary fan bases.

Fortner (1937) suggested that the medial fan probably stabilizes the cephalic fan stem. Others have suggested that it guides particles into the primary fan. However, as it is located ventral and basal to the primary fan, I think that this is unlikely.

Fortner's description of the opening of the cephalic fan, which proceeds as a result of an increase in pressure exerted by the body fluids being forced into the head capsule, is correct. However, I found no evidence of either a basal membrane interconnecting the ventral surfaces of the primary rays or the rotation of Sc_1 , as she described. Fortner illustrated the two lobes of the ventral stem wall but did not describe their movement.

Fortner maintained that the initial extension of the fan is maximal and is subsequently adjusted by an equilibrium between the elastic cuticle of the stem and the force of the current. This equilibrium is controlled by the muscles of the cephalic fan and the muscles of the body. According to her, these muscles are very sensitive to the force of the current and it is through this sensitivity that the larvae detect current variations.

Grenier (1949) and Carlson (1962) found evidence that anatomical differences in the cephalic fans are correlated with feeding differences. However, the difference in the numbers of rays of the three fans in the species studied here is not reflected in feeding habits. Larvae of *S. decorum* have the most rays in each of the three cephalic fans; *C. dacotensis* larvae have the least. The primary fan of *S. decorum* larvae has a small area; the rays are shorter and closer together. The fan thus has a finer 'grid' than that of the other species. In comparison, *C. dacotensis* larvae have fewer rays, a larger fan and a larger 'grid'. Further, the medium larvae of *S. venustum* have more rays than do the large larvae. This apparent discrepancy may be a result either of the measurement of large medium larvae or possibly because medium larvae are adapted to feed more than large larvae. Phelps and DeFoliart (1964) identified two periods of intensive feeding of *S. vittatum* larvae; the first by medium larvae and the second by final instars.

Grenier (1949) described some ecologically important differences in the shape and strength of primary rays of 20 species of blackflies found in France. He concluded that species living in strongly flowing water have shorter, more curved rays composed of stronger cuticle than do species living in moderate currents. He lists *S. venustum* as an intermediate species. As he considered the strength of the cuticle without any special techniques, his conclusions are subjective. Lewis (1953) observed that larvae of species breeding in large breeding sites (larger streams and rivers) tend to have stronger, more flexible primary rays with smaller and thicker basal expansions than larvae of species breeding in small breeding sites. The rays of the various species studied here are structurally similar. However, since preservatives influence the cuticle, interspecific comparisons of cuticle strength were not possible.

The microtrichia of the primary fan rays have attracted much interest. Strickland (1911) claimed that the microtrichia of each primary ray extended to the adjacent ray so that a complete sieve was formed when the primary rays were extended. Fortner (1937) suggested that the microtrichia of the secondary rays have a similar function. Both workers were mistaken. The microtrichia are rarely longer than 1 micron and the rays are about 50 microns apart at their apices. Further, the microtrichia are on the inner curved surface of the ray and not on the side. The microtrichia of any one ray may extend to the adjacent ray but only along the basal quarter of the ray.

Specific differences in the trichiation of the primary rays apparently have no effect on feeding in the species studied here. Rubtsov (1964) claimed that the microtrichia on the primary rays of bloodsucking species are sparse, being 10 to 20 microns apart, and that the microtrichia on the primary rays of non-bloodsucking species are dense, being about 1 micron apart. Yet the three *Simulium* species and *P. fuscum*, *P. fontanatum* and probably *P. multidentatum* are bloodsucking species and these have microtrichia spaced about 1 micron apart or less. *C. dacotensis* is autogenous; the microtrichia are less than 1 micron apart. Thus these species do not support Rubtsov's claim.

Carlsson (1962) reported another ecologically important feature of the structure of the cephalic fans. Larvae of *P. ursinum* Edwards have 24 to 26 large rays and "relatively long 'finer' rays". They are unable to catch bacteria whereas the larvae of *Wilhemia equina* L. have about 46 large rays and "relatively smaller 'finer' rays", which are probably fine enough to catch bacteria. *W. equina* is found in bacteria-rich streams; *P. ursinum* is found in bacteria-poor streams. It is not clear whether Carlsson referred to primary microtrichia or secondary rays as 'finer' rays. The lack of such interspecific differences in the structure of the cephalic fans of the species studied here is probably due to the similarity of their ecological requirements; the three *Simulium* species are all found in the same microhabitat.

The microtrichia of the medial rays of *C. dacotensis* larvae are unique among the species studied. It is doubtful that the medial fan acts as a filter; certainly the sparse microtrichia would be of little help if it did. Although the microtrichia on the primary and secondary rays collect fine particulate debris, the trichiation of the medial ray is probably of little functional importance.

Labrum. — The labrum of *Twinnia biclavata* larvae differs from that of filtering species in that the differentiated labral bristles and their arrangement are adapted for a grazing habit. The dorsal brush of *T. biclavata* larvae may be represented in filtering species by the spindle-shaped patch of spines; in *P. frohnei* larvae this is located posteriorly on the surface of the labrum. The presence of the well-developed, ventral lobe of the labrum in both grazing and filtering species is evidence that the ventral lobe bristles are used for scraping in both types of larvae.

The labral sclerite of *T. biclavata* larvae differs from that of filtering species in orientation of the basal section. The apex of the labral sclerite lacks the lateral blades found in filtering species; this may be a result of the grazing habit. The labral teeth in *T. biclavata* larvae are used in scraping; unlike the labral sclerite of filter-feeding species, the apex of the sclerite projects out from the surface of the labrum (Fig. 41). The sensory labral teeth of filtering species may be lost in *T. biclavata* larvae, or they may be represented by the four medial teeth.

Mandibles. — The mandibles of filtering larvae and of *T. biclavata* larvae represent two forms which are adapted to two modes of feeding. Blackfly larvae which are not typical filterers or grazers, as *Simulium oviceps* Edwards and *Crozetia crozetensis* (Wormersley) larvae, have mandibles intermediate between the two forms. In *T. biclavata* the arrangement of the apical teeth and the stronger development of the flexor muscle are both requirements for scraping. The postantennal buttress of *T. biclavata* larvae, however, is weaker than that of filtering species.

The variation of the arrangement of the mandibular teeth of filtering species has little functional significance. The larger number of teeth of *Prosimulium* larvae may be a primitive feature. The 10 to 12 teeth of *T. biclavata* larvae probably represent undifferentiated apical and inner teeth although their position on the apex of the mandible differs slightly from that of filtering species.

In *T. biclavata*, the brushes which comb the retracted fans of filtering larvae are either lacking or are very poorly developed. Other species without fully developed cephalic fans

have similar reduced complements of brushes. Dumbleton's (1962a) illustrations of the mandible of the larvae of *S. oviceps* and *C. crozetensis* show that they also lack apical brushes and have only poorly developed middle brushes. Davies' (1960) illustrations of the mandible of the first instar of a *Prosimulium* species show a lack of apical brushes. The teeth of the first instar of *Prosimulium* sp. are arranged like those of *T. biclavata* larvae. The basal brushes in all species are the best developed of the mandibular brushes, supporting the suggestion that they aid in the passage of food into the cibarium.

Maxillae. — The shape and arrangement of the brushes of the maxillary lobe of *T. biclavata* larvae are suited for scraping. The apical bristles of the maxillary lobe of *T. biclavata* larvae closely resemble those of the dorsal brush of the labrum which also scrapes the substratum. The palp of *T. biclavata* larvae is similar to that of filtering larvae; the maxillary lobe and the palp of filtering species are all similar.

The role of the curved maxillary spines is unknown. Fortner (1937) suggested that they guide the silk thread. However, this role would be as important in *T. biclavata* larvae as in larvae of filtering species and *T. biclavata* larvae have only very short spines. Further, they are not in suitable position to act as guides for the retracted fans. Work in progress indicates that the apical spine in *Simulium* sp. larvae is sensory (D. A. Craig, *personal communication*).

Labio-hypopharyngeal complex. — The labio-hypopharyngeal complex shows no particular modifications for filtering or grazing. The labial brush probably keeps the silk thread clean and protects the sensory lobes. The role of the lobes of the ligulae is unknown. It is doubtful that they help in the secretion of silk because the *Prosimulium* species examined do not have paired ligular lobes but a group of conical, spine-like bristles and the *Prosimulium* secrete silk as do other species.

Cibarium. — The thickening of the cibarial wall midway along its length probably aids the formation of a bolus prior to swallowing. Fortner (1937) described the movement of food through the pharynx resulting from the contraction of the pharynx. Contraction of circular muscles of the pharynx may contribute to the passage of food in conjunction with the action of the anterior pharyngeal dilators, the cibarial dilators and the labral and mandibular bristles.

Glands. — The function of the dorsal and ventral glands is unknown. Neither Puri (1925) nor Grenier (1949) were able to identify any secretion in the lumen of the glands. Strickland (1911) claimed that the dorsal gland secreted a sticky substance which adhered to the epipharyngeal microtrichia and aided in cleaning the cephalic fan rays. Grenier (1949) suggested that the dorsal gland aided in digestion. He further suggested that the ventral gland is the site of formation of a specialized elastic cuticle required for the movement of the labial lobe within the sheath of the hypostomium. He supported his argument mentioning the occurrence of two similar glandular formations, one at the posterior discs of simuliids and the other at the posterior suckers of blepharocerids. More work is required before the function of the glands is clarified.

Food. — Filter-feeding blackfly larvae ingest any particulate matter of suitable size. The quality and quantity of the gut contents, both nutritive and non-nutritive material found here, are similar to reports in the literature (Chance, 1969). Any differences in food ingested are related to differences in collection localities (Puri, 1925; Grenier, 1949; Jones, 1949a, b, 1950, 1951, 1958; Peterson, 1956). The quantity and quality of food, the quantity of inorganic matter present as well as other environmental factors all influence colonization of water courses by various species of blackflies. However, as long as there is plenty of food available, several species of blackflies may be present in the same stream community (Carlson, 1962). The maximum dimension of measured particulate matter ingested by simuliid larvae ranges from 0.3 to 10,000 microns. The largest particle size is far greater than the

dimension of the cibarium, therefore, the larvae must be capable of ingesting long filaments of food by drawing food through the mouth continuously. The maximum size of globular natural food ingested is approximately 300 microns in diameter. The largest sephadex bead ingested in this study was 345 microns in diameter; the largest size available was 445 microns in diameter. The majority of particles measured, both natural and sephadex, were from 10 to 100 microns in diameter. This size and type of food corresponds closely to those already recorded.

T. biclavata larvae ingest smaller particles; this is probably due to the food available rather than to the limitations of their mouthparts.

The sephadex ingestion experiment shows that the filtering larvae select sephadex only with respect to size. Chemical and physical features other than size have no bearing on the potential of particulate matter for ingestion. This character of simuliid feeding is due to the passive nature of blackfly filtering. Although certain age groups of some species, large *S. decorum* and large and medium *S. vittatum* larvae, ingested a large number of small beads, no trend towards ingestion of any particular size is apparent. Differences in ingestion between age groups within each species is due to the increase in size of the larvae with age. Differences in frequency distribution or mass median diameter of beads ingested by the filtering species studied here cannot be explained by the structural features considered here. More work is required before the effect of the morphology and behaviour on filter feeding of blackfly larvae can be fully described.

The largest diameter of sephadex bead ingested by large *S. venustum* larvae is 185 microns; by medium *S. venustum* larvae, 285 microns. This discrepancy between the size of ingested bead and the size of the larvae may be a result of feeding habits varying with age (Phelps and DeFoliart, 1964). Medium *S. venustum* larvae have more primary rays than do large larvae. However, *S. vittatum* larvae have no structural evidence for differences in feeding with age.

Filter-feeding mosquito larvae ingest particulate matter varying from 7.5 to 165.0 microns. Sizes of particles ingested most commonly by *Aedes fitchii* (F and Y.) and *Culiseta inornata* (Will.) ranged from 15 to 22 microns (Pucat, 1965). *Anopheles messae* Falleroni larvae ingest particles ranging from 22.8 to 34.2 microns in the first instar to 68 to 165 microns in the fourth instar. The diameter of ingested particles is 20% of the width of the head of the first instar and this percentage increases to 31.2% for the fourth instar (Shipitina, 1935, in Bates, 1949). *Culex* sp. ingest particles varying from less than 1 micron to 50 microns in length (Naumann, 1924).

Work done on chironomid larvae indicates that they also ingest smaller particles. *Chironomus plumosus* L. traps all particles above 17 microns and most above 12 microns in its net (Walshe, 1947). The mesh of the net of *Glyptotendipes glaucus* Mg. ranges from 5 to 40 microns (Burt, 1940). Other measurements available for filtering insect larvae are those for trichopterans. Meshes of nets range from 3 by 19 microns in *Macronema* (Stattler and Kracht, 1963) to 50 to 100 microns in diameter for *Hydropsyche* (Kaiser, 1962).

Jørgensen suggests that *Simulium* larvae ingest smaller particles; his assumption is based on the fact that blackfly larvae can be reared on diets consisting only of bacteria (Fredeen, 1960). This diet is not typical for most blackfly larvae.

Mode of feeding

In the earliest reports of larval blackfly feeding, the cephalic fans were described as a current creating apparatus which functioned in a manner similar to that of the mouth brushes of mosquito larvae (Riley, 1870; Osborn, 1896). Strickland (1911) was the first to describe the filtering activity of the fans although there are earlier reports of the mandible cleaning the fans (Osten Sacken, 1870; Miall, 1895). Naumann (1924) and Puri (1925)

gave a more detailed description of feeding and Fortner (1937) gave a comprehensive account of the activity of the fans as well as the transfer of food from the rays to the labrum and mandibles. The filtering mechanism has also been described by Smart (1944), Grenier (1949), Peterson (1956), Anderson and Dicke (1960) and Maitland and Penny (1967) among others.

Filtering is the principal way of feeding for simuliid species having cephalic fans. Although filter-feeding larvae were observed scraping the substratum, they did so only in areas surrounding their posterior discs. When there is sufficient particulate food present in the water, scraping apparently only serves to keep the substratum adjacent to the larvae and the silk pad on which they attach free of debris. Filter feeders do not scrape as rapidly or methodically as *T. biclavata* larvae.

Rubtsov (1964) stated that *Twinnia* larvae filter with their mandibular brushes as well as graze. However, *T. biclavata* larvae were only observed to graze off mats of algae and large clumps of debris as well as the substratum. Yet filter-feeding larvae with the rays of the cephalic fan removed continue normal feeding movements of the mouthparts and particles caught on the labral bristles are transferred to the mouth (Fortner, 1937). *S. oviceps* and *C. crozetensis* larvae filter with abnormal fans as well as graze (Dumbleton, 1962a). These two species probably ingest a size distribution of particles different from typical filtering species.

Reports of cannibalism in the literature are conflicting. Smart (1944) stated that large blackfly larvae eat smaller ones. Badcock (1949) and Maitland and Penny (1967) stated that blackfly larvae occasionally eat members of their own species. Jones (1949b) reported seeing no attacks of cannibalism. However, Peterson and Davies (1960) give a detailed description of a large larva in the laboratory eating first instars, grasping them with its mandibles and forcing them into its oral cavity. Wu (1931) stated that larvae in stagnant water eat dead larvae.

The simuliid cuticle frequently found in the gut is most likely the remains of dead insects or exuvia filtered from the water and cannot be considered evidence of cannibalism. It is possible that late instar larvae may catch and ingest floating first instars, however, there have been no observations or reports to confirm this. Fighting between larvae is not predatory but competition for sites of attachment. A blackfly larva is capable of escaping from a fellow larva before it suffers any lethal wounds.

Filter feeding

Jørgensen (1966) lists three factors on which filter feeding depends: (1) concentration of food available, (2) water flow through the filters whether this is passive as for stream fauna or created by the animal itself, and (3) the efficiency of the filter. Size of particles is a fourth critical factor. Blackfly larvae are typical filterers. This is shown by the influence of current on their feeding, the efficiency (incomplete) of their cephalic fans as filters and their selection of particles by size. Concentration of suspended food may determine whether or not the larvae scrape the substratum to supplement their filtering. There is a minimum concentration of food below which the larvae cannot survive. This level is lower than that required for growth (Carlsson, 1962).

Blackfly larvae at times ingest more than they require. Gut contents apparently may undergo little change as they progress through the alimentary canal. In many cases this is probably due to a high inorganic content of ingested material. Larvae living in streams with large amounts of inorganic material, for example glacial silt in mountain streams, may starve with their guts full of silt. This superfluous feeding reflects the automaticity of filter feeding. In addition, the fact that blackfly larvae catch particles far larger than their mouth orifice is a consequence of automatic feeding.

CONCLUSION

Species of filter-feeding blackfly larvae ingest different distributions of particle sizes. These distributions overlap. None of the structural variation discussed here can explain the differences in ingestion habits. All species ingest particles within a suitable size range whether they may be food or not. Interspecific differences in feeding may be due to differences in behaviour. More work is required before the feeding habits of blackflies can be fully understood.

Feeding differences between filterers and grazers are paralleled by structural variations. The labrum, mandibles, and maxillae of *Twinnia biclavata* larvae are anatomically adapted for grazing; however, the movements of these mouthparts are almost the same as those of the mouthparts of filtering species. The cephalic fans, labrum and mandibles of filtering species are suited for catching and ingesting particles carried by the current.

Blackfly larvae ingest larger particles than other filtering insect larvae. Since filters of insect larvae tend to have a large pore size, especially when compared to those of marine invertebrates (Jørgensen, 1966), blackfly larvae can ingest larger particles than most filter-feeding invertebrates.

The automatic nature of filter feeding by blackfly larvae is a useful tool to increase the selectivity of control against blackflies. Particulate larvicides such as an insecticide adsorbed onto solids which form a suspension when added to streams are the most specific insecticides against blackflies. Grazing species are not pests, and would probably not be affected by such an insecticide since they do not filter feed, but ingest smaller particles than do filter-feeding species.

An insecticide with a particulate formulation in the larger half of the ingested size distribution, 100 to 250 microns for example, would be more readily ingested by blackfly larvae than by other species in the stream fauna. This size range is probably suitable for most species of blackflies, although only later instars would be affected. Because there is not enough information on the feeding habits of other members of the stream fauna, repeated applications may be preferable to a larvicide composed of smaller particles.

Due to the nature of the larval habitat, the exposure time of the larvae to the insecticide can only be a matter of hours. Because of this and because feeding by blackfly larvae is influenced by the concentration of food and current and because larvae tend to prefer particles smaller than 100 microns, enough particulate matter would be required to 'force' the larvae to ingest it by its abundance. The physical character of such an insecticide and its behaviour in streams will have to be carefully investigated before it can be used.

ACKNOWLEDGEMENTS

This work represents part of the requirements of a Master's degree in Entomology. I would like to thank D. A. Craig (University of Alberta) for his supervision and criticism of this manuscript. I am indebted to D. A. Craig and B. Hocking (Department of Entomology, University of Alberta) for their valuable suggestions and encouragement. I am grateful to S. Zalik of the University of Alberta, to M. A. Chance, and my fellow graduate students of the Department of Entomology, University of Alberta, for their advice and assistance.

The following people kindly provided me with larvae: D. A. Craig, D. M. Davies (McMaster University, Hamilton), K. M. Sommerman (Arctic Health Research Center, Anchorage, Alaska), H. E. Welch (University of Manitoba, Winnipeg), and D. M. Wood (Entomology Research Institute, Ottawa). B. V. Peterson (Entomology Research Institute, Ottawa) kindly identified some larvae for me.

I am grateful to the World Health Organization for financing this project, and to the National Research Council and the Ministry of Education, Quebec for further financial support.

REFERENCES

- Abdelnur, O. M. 1968. The biology of some blackflies (Diptera: Simuliidae) of Alberta. *Quaest. ent.* 4(3):113-174.
- Anderson, J. R. and R. J. Dicke. 1960. Ecology of the immature stages of some Wisconsin blackflies (Simuliidae: Diptera). *Ann. ent. Soc. Amer.* 53:386-404.
- Badcock, R. M. 1949. Studies in stream life in tributaries of the Welsh Dee. *J. Anim. Ecol.* 18:193-208.
- Bates, M. 1949. The natural history of mosquitoes. Harper and Row, New York. 378 pp.
- Burton, G. J. 1966. Observation on cocoon formation, the pupal stage, and emergence of the adult of *Simulium damnosum* Theobald in Ghana. *Ann. trop. Med. Parasit.* 60:48-56.
- Burt, E. T. 1940. A filter feeding mechanism in a larva of the Chironomidae (Diptera: Nematocera). *Proc. R. ent. Soc. Lond. (A)* 15:113-121.
- Carlsson, G. 1962. Studies on Scandinavian black flies. *Opusc. ent. suppl.* 21. 279 pp.
- Carlsson, G. 1967. Environmental factors influencing blackfly populations. *Bull. Wld Hlth Org.* 37:139-150.
- Chance, M. M. 1969. Functional morphology of the mouthparts of blackfly larvae (Diptera: Simuliidae). M. Sc. thesis. University of Alberta. 132 pp.
- Chance, M. M. A review of chemical control methods for blackfly larvae (in press *Quaest. ent.*).
- Chaudonneret, J. 1962. Quelques dispositifs remarquables dans les organes de l'ingestion chez la larve de moustique (Diptera: Nematocera). *Annls Sci. nat. Zool.* 4:473-487.
- Chaudonneret, J. 1963. Le problème des 'messoires' des larves de diptères nématocères et la musculature labrale des insectes. *Bull. Soc. zool. Fr.* 88(4):369-378.
- Cook, E. F. 1944. The morphology of the larval heads of certain Culicidae (Diptera). *Microentomology* 9(2):38-68.
- Cook, E. F. 1949. The evolution of the head in the larvae of the Diptera. *Microentomology* 14:1-57.
- Craig, D. A. 1968. The clarification of a discrepancy in the descriptions of maxillary musculature in larval Simuliidae. *Quaest. ent.* 44:31-32.
- Craig, D. A. 1969. The embryogenesis of the larval head of *Simulium venustum* Say (Diptera: Nematocera). *Can. J. Zool.* 47(4):495-503.
- Crosskey, R. W. 1960. A taxonomic study of the larvae of West African Simuliidae (Diptera: Nematocera) with comments on the morphology of the larval blackfly head. *Bull. Br. Mus. nat. Hist. (Ent.)* 10:1-76.
- Davies, D. M. and P. D. Syme. 1958. Three new Ontario black flies of the genus *Prosimulium* (Diptera: Simuliidae). Part 2. Ecological observations and experiments. *Can. Ent.* 90:744-759.
- Davies, L. 1960. The first instar larva of a species of *Prosimulium* (Diptera: Simuliidae). *Can. Ent.* 92:81-84.
- Davies, L. 1965. The structure of certain atypical Simuliidae (Diptera) in relation to evolution within the family, and the erection of a new genus for the Crozet Island black-fly. *Proc. Linn. Soc. Lond.* 176(2):159-180.
- Davies, L. and C. D. Smith. 1958. The distribution and growth of *Prosimulium* larvae (Diptera: Simuliidae) in hill streams in northern England. *J. Anim. Ecol.* 27:335-348.
- Debot, L. 1932. L'appareil séricigène et les glandes salivaires de la larve de *Simulium*. *Cellule* 41:205-216.

- Dumbleton, L. J. 1962a. Aberrant head-structure in larval Simuliidae (Diptera). *Pacif. Insects* 4(1):77-86.
- Dumbleton, L. J. 1962b. Taxonomic characters in the preadult stages of Simuliidae (Diptera). *N. Z. Jl. Sci.* 5:496-506.
- Dumbleton, L. J. 1964. The first instar larvae in the genus *Austrosimulium* (Diptera: Simuliidae). *N. Z. Jl. Sci.* 32:37.
- Fortner, G. 1937. Zur Ernährungsfrage der *Simulium* larve. *Zeitschr. Morph. u. Ökol. Tiere.* 32:360-383.
- Fredeen, F. J. H. 1960. Bacteria as a source of food for black-fly larvae. *Nature, London* 187:963.
- Fredeen, F. J. H. 1964. Bacteria as a source of food for black-fly larvae (Diptera: Simuliidae) in laboratory cultures and in natural streams. *Can. J. Zool.* 42:527-548.
- Fredeen, F. J. H., A. P. Arnason and B. Berck. 1953. Adsorption of DDT on suspended solids in river water and its role on black-fly control. *Nature, London* 171:700-701.
- Fredeen, F. J. H., A. P. Arnason, B. Berck and J. G. Rempel. 1953. Further experiments with DDT in the control of *Simulium arcticum* Mall. in the North and South Saskatchewan Rivers. *Can. J. agri. Sci.* 33:379-393.
- Grenier, P. 1949. Contribution a l'étude biologiques des simuliides de France. *Physiologia comp. Oecol.* 1: 314 pp.
- Hinton, H. E. 1958a. The phylogeny of the panorpoid orders. *A. Rev. Ent.* 3:489-508.
- Hinton, H. E. 1958b. The pupa of the fly, *Simulium*, feeds and spins its own cocoon. *Entomologists mon. Mag.* 94:14-16.
- Hocking, B. and L. R. Pickering. 1954. Observations on the bionomics of some northern species of Simuliidae (Diptera). *Can. J. Zool.* 32:99-119.
- Hora, S. L. 1930. Ecology, bionomics and evolution of the torrential fauna, with special reference to the organs of attachment. *Phil. Trans. R. Soc. Lond.* 218:171-282.
- Jones, T. R. E. 1949a. An ecological study of the River Rheidol, North Cardingshire, Wales. *J. Anim. Ecol.* 18:67-88.
- Jones, T. R. E. 1949b. A further ecological study of calcareous streams in the 'Black Mountain' district of South Wales. *J. Anim. Ecol.* 18:142-159.
- Jones, T. R. E. 1950. A further ecological study of the River Rheidol: the food of the common insects of the main-stream. *J. Anim. Ecol.* 19:159-174.
- Jones, T. R. E. 1951. An ecological study of the River Towy. *J. Anim. Ecol.* 20:68-86.
- Jones, T. R. E. 1958. A further study of zinc polluted River Ystwyth. *J. Anim. Ecol.* 27: 1-14.
- Jørgensen, C. B. 1966. *Biology of suspension feeding*. Pergamon Press, London. 358 pp.
- Kaiser, P. 1962. "Misst" die Köcherfliegenlarve *Hydropsyche angustipennis* Curt. beim Bau des Netzes die Maschenwiete? *Naturwissenschaften* 49:116.
- Kershaw, W. E., T. R. Williams, S. Frost and H. B. N. Hynes. 1965. Selective effect of particulate insecticides on *Simulium* among stream fauna. *Nature, London* 208:199.
- Lewis, D. J. 1953. Simuliidae in the Anglo-Egyptian Sudan. *Revue. Zool. Bot. afr.* 48: 269-286.
- Maciolek, J. A. and M. G. Tunzi. 1968. Microseston dynamics in a simple Sierra Nevada lake-stream system. *Ecology* 49(1):60-75.
- Maitland, P. S. and M. M. Penny. 1967. The ecology of the Simuliidae in a Scottish river. *J. Anim. Ecol.* 36:179-206.
- Matsuda, R. 1965. Morphology and evolution of the insect head. *Am. ent. Inst. Mem.* no. 4. Ann Arbor. 334 pp.
- Miall, L. C. 1895. *The natural history of aquatic insects*. MacMillan and Co., London. 395 pp.

- Naumann, G. 1924. Notizen zur Ernährungsbiologie der limnischen Fauna. *Arkiv. für Zool.* 16(12):1-14.
- Noel-Buxton, M. B. 1956. Field experiments with DDT in association with finely divided inorganic material for the destruction of the immature stages of the genus *Simulium* in the Gold Coast. *Jl. W. Afr. Sci. Ass.* 2(1):36-40.
- Osborn, H. 1896. Family Simuliidae, p. 31-58. *In* *Insects affecting domestic animals*. Bull. Bur. Ent. U. S. Dept. Agric. 5 (n.s.).
- Osten Sacken, R. 1870. On the transformations of *Simulium*. *American Entomologist and Botanist.* 2:229-231.
- Pantin, C. F. A. 1960. Notes on microscopical techniques for zoologists. Cambridge University Press, Cambridge. 76 pp.
- Peterson, B. V. 1956. Observations on the biology of Utah black flies (Diptera: Simuliidae). *Can. Ent.* 88:496-501.
- Peterson, B. V. and D. M. Davies. 1960. Observations on some insect predators of black flies (Diptera: Simuliidae) of Algonquin Park, Ontario. *Can. J. Zool.* 38:9-18.
- Phelps, R. J. and G. R. DeFoliart. 1964. Nematode parasitism of *Simuliidae*. *Bull. Univ. Wis. (Sci. Ser.)*, no. 245. 78 pp.
- Pucat, A. M. 1965. The morphology and function of the mouthparts of mosquito larvae. *Quaest. ent.* 1:41-86.
- Puri, I. M. 1925. On the life-history and structure of the early stages of Simuliidae (Diptera: Nematocera). Part 1. *Parasitology* 17(1):295-337.
- Riley, C. V. 1870. The death-web of young trout. *American Entomologist and Botanist* 2:227-228.
- Rubtsov, I. A. 1964. Simuliidae. Part 14. p. 1-48. *In* E. Lindner, *Die Fliegen der palaearktischen Region* 14:1-48.
- Smart, J. 1944. The British Simuliidae with keys to the species in the adult, pupal, and larval stages. *Freshwater Biological Association of the British Empire. Sci. Publ.* no. 9. 57 pp.
- Snodgrass, R. E. 1959. The anatomical life of the mosquito. *Smithson. misc. Collns* 139. 87 pp.
- Sommerman, K. M. 1953. Identification of Alaskan blackfly larvae (Diptera: Simuliidae). *Proc. ent. Soc. Wash.* 55:258-273.
- Stattler, W. and A. Kracht. 1963. Drift-Fang einer Trichopteran larve unter Ausnutzung der Differenz von Gesamtdruck und statischen Druck des fließenden Wassers. *Naturwissenschaften* 50:362.
- Strickland, E. H. 1911. Some parasites of *Simulium* larvae and their effects on the development of the host. *Biol. Bull.* 21:302-335.
- Walshe, B. M. 1947. Feeding mechanism of *Chironomus* larvae. *Nature, London.* 160:474.
- Walshe, B. M. 1951. The feeding habits of certain chironomid larvae (sub-family Tendipedinae). *Proc. Zool. Soc. Lond.* 121:63-79.
- Williams, T. R., R. Connolly, H. B. N. Hynes and W. E. Kershaw. 1961. Size of particles ingested by *Simulium* larvae. *Nature, London* 189:78.
- Wood, D. M. 1963. An interpretation of the phylogeny of the *Eusimulium*-group (Diptera: Simuliidae) with descriptions of six new species. Ph. D. thesis. McMaster University.
- Wood, D. M., B. V. Peterson, D. M. Davies and H. Gyorkos. 1963. The black flies (Diptera: Simuliidae) of Ontario. Part 11. Larval identification, with descriptions and illustrations. *Proc. ent. Soc. Ont.* 93:99-129.
- Wu, Y. F. 1931. A contribution to the biology of *Simulium* (Diptera). *Michigan Academy of Science, Arts and Letters* 13:543-599.
- Zahar, A. R. 1957. The ecology and distribution of black-flies (Simuliidae) in Southeast Scotland. *J. Anim. Ecol.* 20:33-61.